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PERMEABILITY OF PROTOPLASM TO WATER AND PROTOPLASMIC SWELLING

A. M. Alekseev, G. I. Sovetova and S. D. Samodelkina

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Using the plasmometric method, we have determined the rate of entrance of water into plasmolyzed cells of onion scales (Allium cepa) at various temperatures. If a single vacuolated cell is considered, the permeability of its protoplasm to water will be measured by the amount of water passing through it per unit time per unit surface area at one atmosphere pressure. Protoplasmic permeability may be expressed as: $cm^3/(hr \cdot atm \cdot cm^2)$. Thus, the rate of entrance of water into the vacuole is a measure of protoplasmic permeability.

Onions for the experiment were prepared as follows: Intact onions were first kept for 24 hours at 15° and were then exposed for 24 hours to selected temperatures: 5, 15, 25, 35°. Sections of epidermis from the outer side of the second scale from the top were then prepared. First they were plasmolyzed in 0.8N NaCl, and then deplasmolyzed in 0.2N NaCl. The amount of water which had entered the vacuole during the time required for deplasmolysis (t) was determined by the increase in protoplasmic volume during deplasmolysis: $V_0 - V_1$, where V_0 is the volume of the unplasmolyzed protoplast (equal to the volume of a cell which had lost its turgor), and V_1 is the volume of the plasmolyzed protoplast. The amount of water absorbed by the protoplast during deplasmolysis was calculated on the basis of an over-all time average for the surface area of the plasmolyzed protoplast (S) and an over-all time average for the osmotic pressure gradient. It was necessary to do this since the surface area and the pressure gradient vary during deplasmolysis. The pressure gradient (P) was determined by the difference between the osmotic pressure of a completely plasmolyzed protoplast (P_1) and the osmotic pressure (p) of the deplasmolyzing solution of NaCl, which was 8.4 atm.

TABLE 1

Indices of the Process of Water Absorption by Protoplasm of Onion Epidermal Cells During Deplasmolysis at Various Temperatures

t °C	V_0, μ^3	V_1, μ^3	S, μ^3	t_1, sec	P_0, atm	P_1, atm	P, atm	$\frac{V_0 - V_1}{S \cdot t \cdot P}$ $\frac{\mu^3}{\mu^3 \cdot sec \cdot atm}$
5	90.9	51.0	117.4	561	10.7	19.7	5.6	0.0001071
15	65.3	35.0	84.9	48	11.3	21.1	6.3	0.001159
25	73.2	34.2	96.9	20	11.9	24.2	7.9	0.002532
35	87.1	40.2	107.2	150	10.1	21.9	6.7	0.0004478

The osmotic pressure of a plasmolyzed protoplast (P_1) was calculated from the osmotic pressure of cells in incipient plasmolysis (P_0) and the relative volume of the plasmolyzed protoplast (V_1/V_0).

All necessary measurements and calculations were made in accordance with the instructions of Höfler [1], who originated the plasmometric method of determining protoplasmic permeability.

Data obtained are presented in Table 1.

TABLE 2

Rate of Entrance of Water into the Protoplast (permeability of the protoplasm to water) of Cells of Onion Scale Epidermis at Various Temperatures

t °C	Rate of entrance of water in $\frac{cm^3}{cm^2 \cdot hr \cdot atm}$	Q_{10}
5	$3.86 \cdot 10^{-4}$	
15	$41.71 \cdot 10^{-4}$	10.8
25	$91.14 \cdot 10^{-4}$	2.2
35	$16.12 \cdot 10^{-4}$	−5.6

TABLE 3

Structural Viscosity of the Protoplasm at Various Temperatures

t, °C	C, in units of g
5	646.9
15	708.9
25	1005.0
35	882.6

In Table 1 the rates of entrance of water are expressed as $\mu^3/(\mu^2 \cdot sec \cdot atm)$. These should be converted to a centimeter-hour basis. We will then have final values for the rate of entrance of water into the vacuole, or the permeability of protoplasm to water, at various temperatures. These figures are shown in Table 2.

The phenomenon of plasmolysis is usually discussed on the basis of purely osmotic considerations. Thus, in Academician N. A. Maksimov's Short Course in Plant Physiology (ninth posthumous publication, 1958), it is stated that during plasmolysis "The protoplasm... being semiliquid continues to shrink as a result of a reduction in the volume of cell sap until its concentration is equal to that of the plasmolyzing solution" (p. 45). The protoplasm, consequently, plays an entirely passive role.

Table 1 shows that in our experiments the osmotic pressure of the plasmolyzed protoplast (P_1), that is to say, the concentration of the cell sap, did not reach the osmotic pressure of the plasmolyzing solution (0.8M NaCl), which is 34.2 atmospheres. Thus, the condition of plasmolysis was not characterized by an osmotic equality between the protoplast and the plasmolyzing solution. In our view, the entrance of water into a plasmolyzed protoplast will depend on the ratio of osmotic pressures of the cell sap and the plasmolytic solution only as a first approximation; all those factors which determined the condition of water in the plasmolyzed protoplast, i.e., its partial chemical potential, its activity, must exert an effect on its entrance into protoplasm. The suction pressure in a plasmolyzed protoplast may be expressed as follows: $Sp_A = \mu_{ei} = \mu_1 = RT \ln a_{pA}$, where μ_{ei} represents the over-all partial chemical potential of water in the protoplast, a, its activity, and μ_1, the partial chemical potential of water in the plasmolyzing solution. The symbol pA denotes that in this case water in the cell (and outside it) is at one atmosphere pressure.

The over-all partial chemical potential of water in the protoplasm is determined to a large extent by the osmotic pressure of the cell sap (P): $\mu_{ei} = \mu_1 + V_1 P$. However, it is also influenced by other factors, those on which μ_1 depends. In our opinion, a factor of serious importance is the swelling of plasmolyzed protoplasm. It is responsible for the large temperature coefficient for the entrance of water during deplasmolysis (Q_{10}).

As is well known, the absorption of water during swelling involves several processes: 1) chemohydration of a hydrophilic colloid, and also of dispersed molecules within the colloidal matrix (including the low polymer fraction of the colloidal material); 2) osmotic absorption both into the colloidal matrices and into the intercolloidal liquids containing soluble, low molecular weight polymers and other dissolved materials; 3) immobilization of water by coarse colloidal structures (so-called mechanical or structural binding of water). Chemohydration has a negative temperature coefficient. A decrease of hydration associated with heating must be a consequence of increased thermal motion of the water molecules. Osmotic absorption of water by the colloidal gel during swelling should be increased by heating, since this brings about an increase in the kinetic energy of the particles responsible for the osmotic effect. Although the temperature coefficient of osmosis is positive, it cannot be large (according to the equation, $P = RCT$). However, significantly higher values for the temperature coefficient of swelling as a whole are obtained experimentally. This is accounted for by the fact that heating promotes the rupture of bonds in a colloidal structure and loosens it, thereby heightening swelling. Lipatov [2] writes: "In increasing the temperature, we disrupt apolar bonds and at the same time cause a sharp rise in swelling". In addition to the rupture of apolar bonds there is also a partial destruction of weakly bonded colloidal

structures. With an increase in temperature there is a thermal dissociation of this structure and an increase in disaggregation products in the colloidal gel.* These latter are adsorbed onto the colloidal matrices and because of their solubility reduce the resistance of the elastic gel matrix, i.e., liquefy it. Thus, with heating, swelling may assume a liquefied transmutated character. Let us note in addition that in the case of limited swelling there is only a partial disaggregation of the skeletal matrix of the gel, this being preserved, and the osmotic potential limits the degree of swelling.

Swelling is strongly reflected in the viscosity of the colloidal system. A high viscosity of a layer of highly polymerized material is explained in two ways: 1) by the tendency of long-chain polymers to interact, with the formation of an internal structure or of aggregates (so-called structural viscosity); 2) by the high hydration of long chain polymer molecules. At present the second view is held by only a few workers. In point of fact, the amount of hydration is low, as indicated by calorimetric determinations, being of the order of tenths of a gram of liquid bound to one gram of solid. This amount of bound liquid cannot perceptibly affect the volume of the dispersed phase, and is therefore without effect on the viscosity. The viscosity of highly polymerized colloids is to a significant degree a structural viscosity, because of the aggregation of the colloidal particles. In our opinion, either explanation taken alone is insufficient: Viscosity of highly polymerized materials must be due to the combined effects of aggregation and hydration.

In view of this, it was of interest to determine the effect of heating on structural viscosity of the protoplasm. To do this, we utilized the method of Northen [4]. According to this method, the minimal centrifugal acceleration (C_0) at which protoplasm begins to be discharged from the cells is a measure of structural viscosity. At accelerations below the threshold level, even prolonged centrifugation will not elicit this result. The threshold acceleration (C_0) is calculated according to the formula: $C_0 = \frac{(2\pi n)^2 r}{g}$, where r is the radius of centrifugation, g the acceleration due to gravity, equal to 981 cm/sec^2, and n the smallest number of revolutions at which protoplasm is discharged.

Data obtained are presented in Table 3.

In Table 3 we see that the dependence of structural viscosity of the protoplasm on temperature is expressed by a curve with a single maximum at 25°. It should be pointed out that at low temperatures (specifically, 5°) we were able to determine the structural viscosity only approximately, since heating of the centrifuge interfered with maintenance of this temperature; in this case the figures are somewhat higher.

How is the temperature curve for structural viscosity to be explained? In our opinion, it is dependent on the ratio between the strength of hydration and the attractive forces between the hydrophobic radicals of the protein molecules. With an increase in temperature, the degree of hydration is reduced, while the attractive forces between molecules become more important; there is a molecular aggregation and an increase in the structural viscosity of the protoplasm. At a high temperature, protoplasmic swelling assumes a liquefied transmutated character which is accompanied by a partial disruption of structure and a fall in structural viscosity.

If we compare the data of Table 2 with those of Table 3, we see that the temperature curve for the rate of passage of water into the protoplasm corresponds with that for structural viscosity of the protoplasm; in both cases there is a single maximum at 25°. This circumstance may be explained on the assumption, proposed by one of us (Alekseev [5]), of mesoplasmic swelling and shrinking during passage of water into the vacuole. The entrance of water into a cell along an activity gradient results in mesoplasmic swelling, in an increase of weakly bound or even free water. Insofar as this swelling is limited in nature, the forces of attraction between protein molecules set the limit. The pressure created by these forces raises the partial chemical potential (activity) of water in the mesoplasm in comparison to that of the water in the vacuole; as a result, part of the water in the mesoplasm moves into the vacuole, and the mesoplasm shrinks. Mesoplasmic shrinkage induces the uptake of more water from the external environment, etc. In this process the polarity of the outer protoplasmic layer must be kept in mind (Sabinin [6]). A definite orientation of molecules in this layer makes it differentially permeable to water in opposite directions. As a rule, it is less permeable to water moving out of the cell than to water moving into it.

* Disaggregation may greatly increase the Q_{10} for the osmotic pressure of a hydrophilic sol.

Insofar as mesoplasmic shrinking is related to the action of attractive forces between structural elements of the protoplasm, the increase in a known temperature range of both structural viscosity and protoplasmic elasticity must increase the rate of passage of water into the vacuole. Colloidal dehydration by heating must also have this effect. With further increase in temperature, however, protoplasmic swelling becomes transmutagenic in nature, and is accompanied by the disaggregation of structural elements and a decreased protoplasmic elasticity; consequently there is a reduction in the rate of passage of water into the vacuole at this high temperature.

SUMMARY

On the basis of our investigations we conclude that movement of water from the external medium through the protoplasm into the cell sap is not a purely osmotic process, since it is related to swelling and shrinking of the mesoplasm, and to changes in its structural viscosity. This circumstance explains the relatively high temperature coefficients of the permeability of protoplasm with respect to water.

LITERATURE CITED

[1] K. Höfler, Jahrb. wiss. Bot. 73 (1930).

[2] S. M. Lipatov, Highly Polymerized Compounds (Lipophilic Colloids) (Izd. AN BSSR, 1943) [In Russian].

[3] S. M. Lipatov, Physical Chemistry of Colloids (Goskhimizdat, 1948) [In Russian].

[4] H. Northen, Protoplasma, 31 [Cited by Belousova, Bot. Zhur. 42, 7 (1957)].

[5] A. M. Alekseev, Problems in Botany, 1 [In Russian] (Izd. AN SSSR, 1950).

[6] D. A. Sabinin, Physiological Bases of Plant Nutrition [In Russian] (Izd. AN SSSR, 1955).

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HARDENING OF NORTHERN WOODY PLANTS BY TEMPERATURES BELOW ZERO

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Effective study of the phenomenon of plant frost hardening was begun late, about forty years ago. It became feasible only after the necessary groundwork was laid. The elucidation of the properties of ice formation inside plants [1-3] was of great importance in this regard, as was the establishment of the effect of protective materials on cell frost resistance [4-6]. Finally, investigations on plant hardening were made only after physiologists had begun to use cold techniques in the laboratories, exposing the experimental material to the effect of various low temperatures for selected lengths of time.

At first cabbage was used in hardening studies [7, 8], then winter cereal grains [9, 10], and most recently woody plants [11-13]; i.e., workers shifted from weakly resistant to more resistant material. Originally, the main attention in our laboratory was directed to the first stage of hardening, which occurs at above-zero temperatures. In the course of these studies the external conditions required for hardening were determined [14], as well as the physiological changes induced by such conditions: suppression of growth, decrease in cellular auxin levels [15, 16], accumulation of sugars [17], etc.

In the study of hardening at below-zero temperatures (second stage), it is necessary to deal with frozen material, which creates technical difficulties. We were able to carry out such studies only after the opening of the Experimental Climate Station [18-20]. Northern woody species proved to be suitable experimental material, since they develop a very high frost resistance after the second stage.

First of all, observations on the hardening process during winter under natural conditions were made. For a period of several years the frost resistance of one- and two-year-old shoots cut from a tree at various times during the year was determined. In order to prevent passage through the second stage in the cold chamber, chilling was carried out relatively rapidly, beginning at the temperature prevailing the day of removal of the branches. In this way, the frost resistance dynamics of apple, fir, birch and other woody species were determined under conditions in the Moscow region. During the growth period, shoots die at temperatures just below -5°C (Fig. 1). In September an increase in frost resistance occurs and it becomes particularly marked after the appearance of frosts. The greatest frost resistance was found in the most severe winter period.

Here it proved to be true that even severe frosts increase the resistance of woody plants. Thus, those frosts which injure and kill plants during their growth period become beneficial after the first hardening stage, being utilized by the organism to increase its resistance. The character of a plant's reaction to external conditions is thus sharply altered after its physiological transformation. During this hardening, which was observed in the conditions of Moscow, branches were capable of withstanding without significant injury the following temperatures: in Grushovka moskovskaya apple, down to -40°C, in fir, to -50°C, and in birch, to -65°C. Even in a single tree the maximum resistance varied from year to year; for example, in birch it fluctuated between -50 and -65°C; in cold winters it was higher than in mild winters.

Observations made during the development of frost resistance in the field led subsequently to attempts to learn by laboratory experiments what below-zero temperatures are required for successful completion of the second hardening stage. It was found that in northern woody species frost resistance, which is usually observed

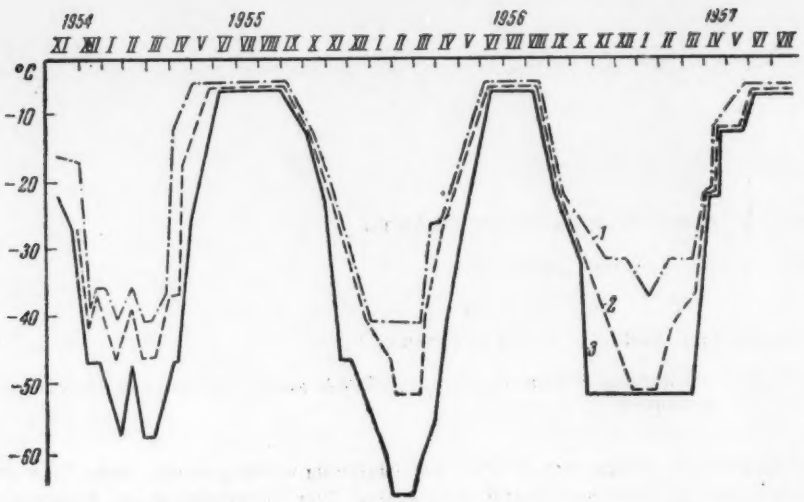

Fig. 1. Seasonal changes in frost resistance of woody plants over a three-year period. 1) Slavyanka apple; 2) common fir; 3) warty birch.

Fig. 2. Frost resistance of branches of Grushovka moskovskaya apple. On the left, two branches which withstood exposure to -60°C temperatures after hardening in the laboratory; on the right, two branches which died at -40°C after hardening outside in the winter in the Moscow region.

Fig. 3. Frost resistance of black currant shoots cut in winter. 1) Control (without laboratory hardening); 2) after hardening outside in the Moscow region, died at -45°C; 3) after laboratory hardening, survived -195°C (new roots formed on the lower portion of the shoot).

under natural conditions, is far from maximal. By means of supplementary hardening in our laboratory, N. N. Khvalin succeeded in increasing the frost resistance of one- and two-year-old branches of Grushovka moskovskaya apple to such an extent that after exposure to -60°C for twenty-four hours they opened their buds and flowered (Fig. 2). In gardens this apple, as already indicated, died between -40 and -45°C. This variety does not overwinter in Siberia. Such a comparison makes it clear that in nature conditions for the development of the second hardening stage are less favorable.

Northern forest trees develop a still higher resistance after supplementary hardening in the laboratory. Fir branches removed after a thaw died at -30°C. Branches taken at the same time from the same tree, after a

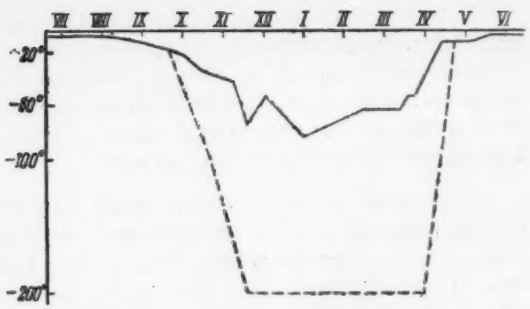

Fig. 4. Seasonal changes in the frost resistance of warty birch branches. Solid line) after hardening outside in the Moscow region; interrupted) after laboratory hardening. From May to October the capacity to undergo hardening at below-zero temperatures is absent.

laboratory hardening withstood -70°C; even at -100°C only minor injury was incurred, while at -195°C extensive injuries resulted. One- and two-year-old pine and birch shoots exhibited a higher resistance after a laboratory hardening. After exposure to -195°C their buds developed, leaves were formed, and no significant injuries were suffered. Of orchard plants, black currant shoots also withstood the extreme temperatures indicated without marked injury (Fig. 3).

The Japanese worker Sakai was able to increase the frost resistance of poplar and ivy grafts so that they withstood -195°C for 20 days.

Thus, it was shown that northern woody species are capable of withstanding exposure to the lowest temperatures which might be encountered in nature, and even lower ones.

The extreme frost resistance indicated for northern woody species was attained in our laboratory by a slow stepwise cooling. Shoots removed in the winter were exposed for a twenty-four hour period to selected temperatures: -10, -20, -30, -40, -50, and -60°C. Laboratory experiments on the second hardening stage enable us now to answer the question — what prevents woody plants from attaining high frost resistance? Sharp fluctuations in winter temperatures are one such unfavorable factor. In agricultural practice, the danger of winter thaw has long been known. Our experiments show that not only thaws but also sudden frosts are harmful.

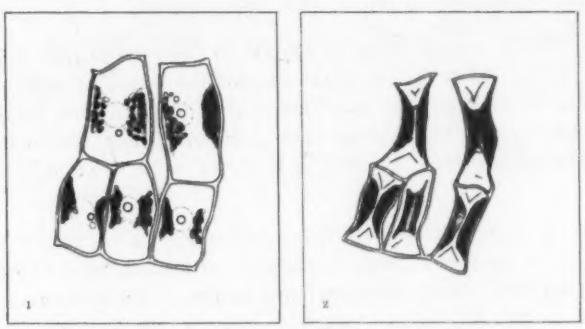

Fig. 5. Parenchyma cells in a longitudinal radial section of a fir needle. Magnif. 680, Dec. 26, 1956: 1) before freezing; 2) after freezing, at -40°C; cell volume has decreased, and the intercellular spaces have increased in size; magnif. 680 (Dec. 2, 1956).

It should not be thought, however, that only the second stage is important in the development of resistant plants. Branches removed from the tree from May to September cannot be made resistant even if subjected to the most favorable regime of below-zero temperatures (Fig. 4). It is necessary that the protoplast be first conditioned in an appropriate manner. Only then is the plant able to utilize frosts, including severe ones, for protection against death by freezing.

In what manner are plants able to withstand such extremely low temperatures as -195°C? What takes place in the frozen organism during the second hardening stage? This stage has seemed mysterious in the past. A possible approach to the explanation of the mechanism of frost resistance is by microscopic observations during freezing and death of sections. In our laboratory, Krasavtsev [21] and G. A. Samygin have constructed freezing microscope stages which enable the observer in a warm room to examine sections at any desired temperature down to

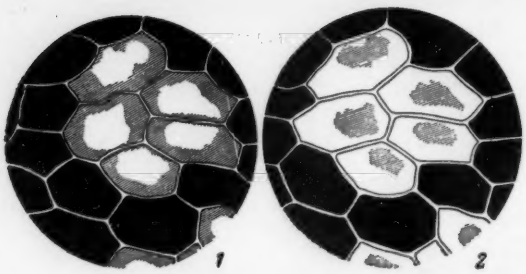

Fig. 6. Cross section of a fir needle in winter time. Fluorescence of cells in blue light. 1) Before freezing, bluish-gray fluorescence of cell sap, bright red fluoresecnce of chloroplasts; 2) at -55°, after death, a bright yellow fluorescence of the whole cell, chloroplasts brownish. Cells injured in section preparation do not fluoresce (black).

- 80°C. An ultraviolet microscope was used in these observations. Cellular fluorescence improves the visibility of cellular contents and after the formation of ice facilitates the detection of protoplasmic death. Most of the observations were made with sections of fir and subsequently confirmed with other material.

During the cooling of tissues hardened under natural conditions their cells remain transparent, and beginning at -8 to -12°C gradually shrink (Fig. 5). At the same time the intercellular spaces become larger. Plasmolysis after thawing shows that the cells are still living.

Microscopic observations lead to the conclusion that with slow cooling of hardened tissue ice is always formed first outside the cells. Water moves out of them and freezes in the intercellular spaces. At the same time, the protoplast is strongly dehydrated. In shrinking it undergoes a marked mechanical strain. This may be inferred from the increase in the size of intercellular spaces and the tearing of cells from one another. The hardened protoplast withstands all these favorable effects without injury.

Observations in blue and ultraviolet light confirm this conclusion. At above-zero temperatures, cells of hardened fir needles exhibit two fluorescences: one, due to chlorophyll, is red, and the second is gray from some other unknown natural fluorochrome (Fig. 6). Cells injured during preparation of sections show almost no fluorescence; chloroplast fluorescence is very weak, and the other fluorochrome is not visible, being represented only by a dark area. This property makes it possible to direct observations to normal protoplasm.

If the temperature of the microscope stage is reduced, to -21°C for example, the fluorescence of living cells is somewhat altered: that of chlorophyll is weakened, and the other fluorochrome becomes almost white; with further cooling the fluorescence becomes more intense (Fig. 7). After cell death it changes from white to yellow in the frozen sections (Fig. 6). Upon further cooling the intensity of yellow fluorescence increases, and at -55°C and -65°C becomes brilliant (Fig. 7). Judging from this, these tissues die in the process of freezing, and not during thawing.

Microscopic observations show that from -40°C to -65°C and even to -80°C there is no further perceptible shrinking of the fir needle cells, but they become darker and less transparent and dark streaks appear (Fig. 7). All this leads to the supposition that during this time ice is forming in the protoplasm. Such cells cannot be plasmolyzed after thawing. Microscopic observations also indicate that the tonoplast in hardened cells is relatively more resistant than the remainder of the cytoplasm. In fir, the tonoplast remains intact in the winter down to -65°C (Fig. 7). The surviving tonoplasts usually die after two days at room temperature. Plant resistance is determined by cytoplasmic properties, and not those of the vacuole.

With rapid cooling the cells freeze in a different manner. According to microscopic observations, ice forms even at -15°C, not outside but inside the cells, as shown by their darkening and by the shift from white to yellow fluorescence (Fig. 7). Cells freeze rapidly one after the other, and their volume remains unchanged, or even increases. After this such cells, in which ice has formed, are not capable of being plasmolyzed; they are always dead. With rapid cooling the tonoplast also dies, as a rule.

Thus, even hardened cells are adapted only to a relatively slow cooling. If cooling is rapid, water is unable to pass into the intercellular spaces, and this leads to extensive supercooling and subsequently to the appearance of ice in the protoplast, which is always fatal. From this it follows that hardening does not make colloids of the protoplast resistant to the formation of ice in it. Survival of cells in frost resistant plants is achieved only by a timely dehydration as a result of the freezing of water in the intercellular spaces.

Results of microscopic observations obtained in our laboratory lead to the following conclusions. Hardened cells of frost resistant woody species are well protected from ice formation in the protoplasm. In naturally hardened

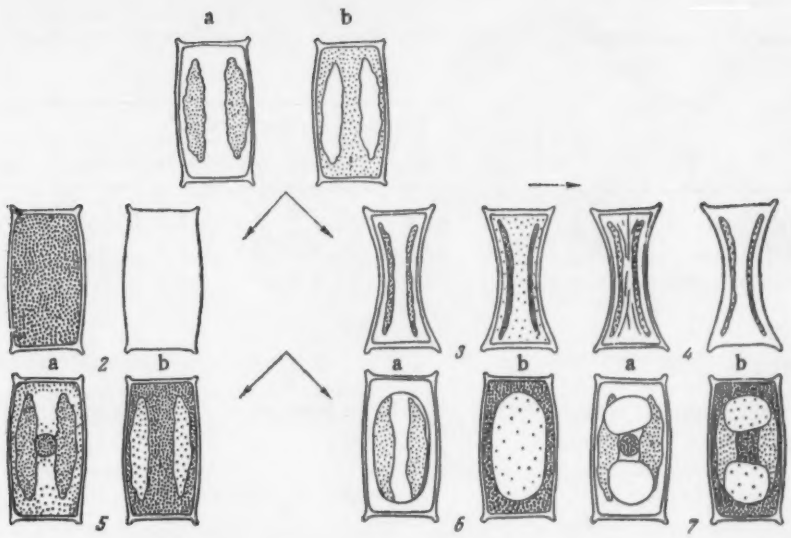

Fig. 7. Diagrammatic sketch of the parenchyma cell of a fir needle. Observations were made during cooling and also after thawing. 1) Before freezing: A)in white light, B) fluorescence in blue light, bluish gray for cell sap, bright red for chloroplasts; 2) rapid freezing to -25°C and lower, ice formed in the cells. Cell volume is not decreased; fluorescence in blue light, bright yellow throughout the cell; 3) gradual freezing to -35°C. Cell volume is decreased; fluorescence in blue light, white from the cell sap; 4) gradual freezing to -65°C and lower, fluorescence in blue light, bright yellow throughout the protoplast; 5-7) after thawing and exposure to a plasmolyzing solution; 5) no plasmolysis, cell dead; 6) plasmolysis occurring, cell living; 7) plasmolysis of the tonoplast, protoplasm dead.

fir this is observed at about -40°C. With slow cooling, water can flow out of the cells and freeze in the intercellular spaces. The hardened protoplast proves to be able to withstand both severe dehydration and mechanical strain. Death occurs only after further cooling, when the cell volume abruptly ceases to decrease. The Japanese worker Asahina [22] observed the same behavior of cells. This phenomenon may be understood if it is assumed that as a result of a suppression of water flow out of the cell supercooling occurs. When supercooling reaches a significant level and a considerable amount of supercooled water accumulates, it freezes inside the protoplast and disrupts the submicroscopic structure. To explain the death by freezing of hardened cells in terms of dehydration or mechanical deformations is not feasible, since death in the course of further temperature reduction occurs after cell volume ceases to decrease markedly.

The mechanism of death of plants in a growth phase or a weakly hardened condition has been less studied. In this case many cells die even without ice formation in the protoplast, according to G. A. Samygin. In order to understand how this happens, further studies are required.

Determination of the amount of unfrozen water in plants at various temperatures offers another approach to the study of the second hardening phase. Of the existing methods, the calorimetric method, based on dry weight, is considered the most promising. It has usually been applied down to -20°C and occasionally to -45°C [23]. We were able to make determinations at very low temperatures as well; -100°C and even -115°C. In this case it was necessary to make corrections for the heat of swelling and solution, and for the heat capacity of hygroscopic water. In spite of these corrections, determination of the amount of ice in plants during extensive cooling may involve a whole series of inaccuracies which may affect absolute values, but relative data on various treatments are compared.

The amount of unfrozen water in one-year-old birch branches cut in the winter was determined after they had been kept for a twenty-four hour period at the following temperatures: -10, -20, -30, -40, -50, -60, and -100°C.

TABLE 1

Content of Unfrozen Water in One-Year-Old Birch Branches at Various Temperatures Below Zero (in % of dry wt)

Treatment	Hardening temperature in °C						
	-10	-20	-30	-40	-50	-60	-100
Initially killed with steam	26.4	22.6	10.6	8.4	8.0	7.2	7.0
After laboratory hardening	34.2	23.5	12.2	9.0	8.7	7.6	6.9
Percent of supercooled water	+7.8	+0.9	+1.6	+0.6	+0.7	+0.4	-0.1

TABLE 2

Content of Unfrozen Water in One-Year-Old Pine Branches at Various Temperatures Below Zero (in % of dry wt)

Treatment	Cooling temperature, °C			
	-20	-40	-60	-100
Initially killed with steam	27.7	20.8	17.3	12.3
After laboratory hardening	28.3	20.8	19.9	12.0
Percent of supercooled water	+0.6	0.0	+2.6	-0.3
First stage only	40.5	35.1	24.6	[11.6]
Percent of supercooled water	+12.8	+14.3	+7.3	-0.7

Note: In this and succeeding tables, the figures for plant injury by frost are underlined; for those which were killed by frost the figures are completely enclosed.

TABLE 3

Content of Unfrozen Water in One-Year-Old Fir Branches at Various Temperatures Below Zero (in % of dry wt)

Treatment	Cooling temperature, °C					
	-10	-20	-30	-40	-50	-60
Initially killed with steam	32.7	14.2	15.9	12.1	8.7	6.8
After laboratory hardening	45.9	27.1	22.0	13.5	8.8	6.5
Percent of supercooled water	+13.2	+12.9	+6.1	+1.4	+0.1	-0.3

With this treatment the second hardening stage was completed, and the plant material withstood without injury a temperature of -195°C. This treatment was compared with another in which branches cut at the same time were killed with steam and then subjected to a twenty-four hour cooling period at the temperature given above Table 1).

The figures of Table 1 indicate that the content of unfrozen water in the shoot steadily declined with a decrease in temperature. During severe freezing the cells were dehydrated to such an extent that only hygroscopic water remained, and even that was gradually converted into ice. Although the protoplasm becomes air-dry with this treatment and undergoes a sharp reduction in volume, the branches are still alive after thawing, and produce new leaves from their buds. Since they survived even at -195°C, it must be concluded that after the second hardening stage the protoplast is able to withstand not only almost complete drying, but also the associated mechanical deformation. It is also evident that frost resistance in northern woody species is not based on the water-retaining capacity of the cells. The organization of the protoplasm even after hardening does not prevent the water present from crystallizing in the intercellular spaces.

TABLE 4

Content of Unfrozen Water in One-Year-Old Oak Branches at Various Temperatures Below Zero (in % of dry wt)

Treatment	Cooling temperature, °C		
	-20	-40	-51.5
Initially killed with steam	39.2	6.1	3.7
After laboratory hardening	42.1	20.0	3.4
Percent of supercooled water	+2.9	+13.9	-0.3
First stage only	43.1	7.2	–
Percent of supercooled water	+3.9	+1.1	–

TABLE 5

Rate of Flow of Water from Cells of Birch and Fir Kept for Various Lengths of Time at -20°C (unfrozen water in % of dry wt)

Experimental material	Exposure time, hr				
	2	6	24	24	48
Birch	29.2	28.4	27.2	22.1*	25.9
Percent of supercooled water	7.1	6.3	5.1	–	3.8
Fir	56.7	46.4	21.3	12.9*	–
Percent of supercooled water	43.8	33.5	8.4	–	–

* Figures for branches killed with steam

TABLE 6

Comparative Rates of Dehydration and Hardening in Birch and Fir at -10°C

Item measured	Hardening time, in hr						Killed with steam
	0	3	6	12	24	48	
Birch							
Frost resistance	-40°	-40°	-40°	-40°	-50°	-60°	–
Percent of unfrozen water	–	39.7	35.3	34.6	34.2	33.2	26.4
Fir							
Frost resistance	-30°	-30°	-30°	-30°	-40°	-40°	–
Percent of unfrozen water	–	69.1	50.8	47.7	45.9	45.9	32.7

It is interesting to compare amounts of unfrozen water in branches which had completed the second hardening stage and in branches which were initially killed. In this manner data on the permeability to water of the hardened protoplast during severe freezing can be obtained. Until now, such determinations have been made only at temperatures above zero [24]. Moreover, in the development of a theory of frost resistance it is useful to know the magnitude of protoplasmic permeability in the frozen plant. We obtained this information in the following manner.

TABLE 7

Aftereffect of the Second Hardening Stage in Various Woody Plants (frost resistance, °C)

Experimental material	Original frost resistance after 26 days' hardening	Frost resistance after thawing and exposure to +2°C for (hr):				Frost resistance under natural conditions at the beginning of Nov.
		6	24	48	168	
Birch	-195	-45	-40	-35	-30	-25
Pine	-195	-45	-45	-40	-30	-30
Fir	-100	-40	-40	-30	-20	-20
Oak	-60	-40	-35	-20	-20	-20

First, it was established by microscopic observations that in well hardened branches of northern woody species ice is always formed in the intercellular spaces. For this to occur, water must be able to pass out of the protoplasm. By determining calorimetrically the amount of ice which had been formed in the shoot in a definite period, we obtained data relating to protoplasmic permeability. We always controlled the experiments so that the material was not injured after freezing. In such experiments it was found that all the ice was extracellular. For our purposes, relative values for the permeability of hardened protoplasts were sufficient. We shall compare living shoots with those killed with steam Table 1). The figures for the two cases prove to be similar. For living shoots they were 0.4-1.6% higher than for dead ones. This is true with the exception of -10°C, at which a marked supercooling occurred in the protoplast. Thus, it is experimentally shown that in severe freezing conditions the protoplasts of well hardened cells actually have a high permeability to water, which passes out of them with almost the same ease as out of dead cells.

In order to clarify the role of the second hardening stage, a third treatment was employed in one of the experiments with pine branches; here the experimental material had completed only one stage. The shoots, removed from the tree in winter, were thawed at +1°C. After this they were immediately exposed to temperatures of -20, -40, -60, and -100°C.

Even in pine branches which had undergone the second hardening stage and survived exposure to -195°C, there was almost the same amount of unfrozen water as in branches previously killed (Table 2).

Only the treatment in which the second hardening stage is omitted gives markedly different results. In this case, the amount of unfrozen water at -20 and -40°C is much higher — from 13-14%; with further reduction of temperature to -60°C, injuries were incurred and the amount of unfrozen water dropped to 7%. At -100°C, when the branches had all died, the figures are comparable to those for branches killed with steam. A gradual fall in the curve of water content is explained by the fact that different cells and tissues die at different times; in some death occurs at higher temperatures than in others. A comparison of treatments indicates that the second hardening stage actually ensuresthat almost all the water which can freeze at a given temperature will pass into the intercellular spaces. It is of importance that an increased content of unfrozen water was observed only in shoots less resistant to frost, which had completed only the first hardening stage. It is striking that supercooling occurred before injury to the tissues. All this indicates that the increase in the percent of unfrozen water is due not to its binding, but to supercooling.

It is of interest that there are two types of supercooling in plants. One occurs before freezing of the organism, when there is as yet no ice. The other occurs shortly before death, when there is already much ice in the intercellular spaces, but the cells remain unfrozen. To a point such supercooling is beneficial, since cells are protected from a freezing which is severe for them.

In less frost resistant species the permeability of the protoplast at certain temperatures proves to be insufficient; in these cases there is supercooling in the protoplast. For example, in fir shoots which had completed the second hardening stage in the laboratory, water was unable to pass out of the cells in the course of twenty-four hours at temperatures from -10 to -30°C (Table 3).

This table shows that water which had been accumulated at these temperatures could later pass out of the cells during exposure to lower temperatures, such as -40°C. These data indicate that at -10°C to -30°C the conditions for passage of water out of cells were less favorable. Therefore, in hardening it is necessary to keep weakly resistant species at these temperatures longer than twenty-four hours.

In less resistant woody species not only supercooling of water, but also freezing, could be observed inside the cells (Table 4).

For example, in oak branches which had been hardened in the laboratory, there was little supercooling in the cells at -20°C (about 3%), but at -40°C the water content rose to 14%. At -51.5°C it was absent, but the branches were already extensively injured. Such behavior may be explained as follows. Down to -20°C the permeability of the protoplast was still sufficiently high, while at -40°C it had dropped sharply, and supercooled water had accumulated in the cells which, with more extensive cooling, froze in the cells. When shoots which had not passed through the second hardening stage were exposed to a rapid reduction in temperature, water could not pass out of the cells even with light freezing. In this case many cells died at -20°C, and therefore the level of supercooling was not high.

The existing data are in agreement that, at the beginning, ice is usually formed in the intercellular spaces. With a further temperature reduction, conditions for flow of water out of the cells may deteriorate, with a resultant supercooling of the protoplast. The more thoroughly the plant is hardened, the less likely is it that insufficient permeability will occur during more severe frosts; indeed such a situation may not arise. In that case plants which survive freezing are obtained. The timely dehydration of the protoplast protects it from the extensive formation of ice crystals. In our laboratory the employment of a suitable hardening regime made it possible to develop frost resistance in birch, pine, and black currant to the extent that they withstood without injury exposure to -195°C.

In order to determine how rapidly water passes out of cells of frozen plants at different levels of frost resistance, measurements were made of the amount of unfrozen water in birch and fir branches kept at -20°C for 2, 6, 24, or 48 hours. Previously killed branches served as controls. Data obtained are presented in Table 5.

Table 5 shows that in birch, the most frost resistant species, water flowed out of cells very rapidly. The greater part of it passed into the intercellular spaces in the first two hours. The small quantity of supercooled water which had remained (7%) subsequently passed out more slowly; even after 48 hours this process had not been completed. In the less resistant fir, after the first two hours there still remained in the cells a large amount of supercooled water (about 44%), this amount being sharply reduced only after 24 hours. Even then there was still 8% supercooled water remaining. A comparison of birch and fir shows that in frost resistant forms the cells are able to become dehydrated very rapidly in freezing weather as a result of ice formation in the intercellular spaces. This explains why resistant species can survive more extreme fluctuations in winter temperatures.

The data from our laboratory show that the role of the second hardening stage does not consist solely in the prevention of supercooling in the cells. It is sufficient to compare in our parallel experiments the rate of dehydration with the rate of hardening (Table 6). For example, birch branches were hardened at -10°C for various lengths of time — from 3 to 48 hours. The experiments showed that an increase in resistance was observable after 24 and 48 hours at this temperature. By parallel calorimetric determinations it was shown that after 12, 24, and 48 hours the percent of unfrozen water in the cell had not changed markedly (0.4% and 1% in all). Therefore, hardening is a more lengthy process than is that of ice formation. After dehydration the protoplast must still have time for physical change of some sort. That this is not fortuitous can be shown by experiments with fir branches. In this case an increase in resistance was also found after 24 hours. Even here the content of unfrozen water changed very little, 1.8%, with an increase in the hardening period from 12 to 24 hours.

Some fundamental physical changes in the properties of the protoplasm take place during the second hardening stage, after dehydration has proceeded to a significant degree. This is indicated by an after-effect from the second stage. Experiments show that as early as six hours after thawing there is a fall in resistance of birch and pine, for example, from -195 to -45°C (Table 7).

Upon further exposure of the plants to a temperature of +2°C, at which the first stage is not reversed, there is a further decline in resistance of 15-20°C. A similar phenomenon occurs in fir and oak. If the reason for survival were simply a single dehydration at low temperature, then after reabsorption of the melted water the cells would have returned to the level of resistance which is found after the first hardening stage, this level being achieved

under natural conditions at the beginning of November. Actually, however, the resistance during the first and second days proves to be markedly higher, and only later does it fall to the expected level. These data provide evidence that at low temperatures, in addition to a gradual dehydration, there are some physical changes in the protoplasm, the effect of which is not immediately lost after reabsorption of the melted water.

From these data it follows that the duration of the second hardening stage is determined by the time required not only for the flow of water out of cells but also for a definite alteration in the submicroscopic structure of the protoplasm. The less frost resistant the plant, the more slowly this stage progresses. Some plants require a month to complete it. The critical factors in this stage are, first, dehydration, which causes a reduction of intermolecular distances, and second, low temperatures, which decrease the thermal motion of molecules. By the action of these two factors, a condition of orderly molecular arrangement is created and maintained. As a result, a definite submicroscopic protoplasmic structure is created which gives rise to such new protoplasmic properties as high permeability to water and resistance to dehydration and mechanical deformation. This transformation becomes possible only after preconditioning of the cells at the end of the summer and in the fall. Hardening ensures that cells of frozen plants will be free of ice even at extremely low temperatures. This is a working hypothesis of the mechanism of the second hardening stage which must still be proven and extended.

In the improvement of conditions for completion of the second hardening stage in the field, the protection of plants from sharp fluctuations of temperature is of great significance. For winter crops this protection is afforded by a layer of snow. For fruit trees, a slower cooling is obtained by planting in zones shielded from the wind; another advantage of this practice is that the supercooled condition is more easily maintained and branches are not subjected to mechanical stress. In areas with sunny weather during the cold period, the trunks and crowns must be protected from overheating; temperatures may reach 10° and sometimes 30°. This can be achieved by whitewashing, by covering the lower stems and crotches with snow, by grafting stems onto more resistant varieties, and by other methods. The attention of selective breeders should be concentrated on the development of new forms resistant to sharp fluctuations in temperature.

SUMMARY

The greatest resistance of branches of northern woody plants is observed during the most severe winter periods. After hardening under natural conditions of the Moscow region the Grushovka moscovskaya apple tree is capable of withstanding temperatures down to -40°C, fir trees down to -50°C and birch trees down to -65°C. The frost resistance of apple trees can be raised to -60°C, of fir to -70°C and of pine, birch and black currant to -195°C, by additional laboratory hardening (slow stepwise cooling). Trees are found o be susceptible to this type of hardening only between October and May. Rapid changes of temperature in the winter, e.g., thaws or sudden frosts, impede the development of a high frost resistance.

Microscopic observations on freezing and destruction by frost of tissue sections showed that upon slow cooling, ice in the hardened cells is always produced outside the cells, the protoplast in this case being strongly dehydrated, and the cell volume sharply diminished. With further approach to dangerous temperatures no further shrinkage of the cells is noticeable, but they darken, and this indicates that ice formed within the protoplast. After thawing such cells do not plasmolyze.

Upon rapid freezing at -15°C or lower the ice is formed inside the cell, not outside it. This can be inferred from the yellowing of the cells and change of white fluorescence under ultraviolet to a yellow fluorescence. Such cells are always dead. Cells are adapted only to relatively slow cooling. Hardening does not render the protoplast colloids stable with respect to formation of ice inside it.

A calorimetric determination of the amount of ice in the tissues of stable woody plants carried out at various temperatures down to -115°C showed that the amount of unfrozen water in shoots dropped continuously as a result of additional laboratory hardening, and in severe frosts only hygroscopic water remained. Some objects withstood -195°C, at which temperature complete drying was observed.

Hardening at negative temperatures ensures the flow to intercellular spaces of almost all the water which may freeze at the given temperature. By comparing the amount of water which did not freeze in branches given supplementary laboratory treatment with that in branches which were initially killed, it could be shown that water flows from hardened protoplasts at almost the same rate as from killed ones. It must therefore be concluded that frost resistance of northern woody plants is not based on the water retaining capacity of the cells.

Comparison of drying and hardening rates shows that the latter is more prolonged. The hardening time depends not only on the time required for flow of the water from the cells but also for a definite reorganization of the submicroscopic structure of the protoplasm. The active agents in this case are, first, dehydration, which leads to a decrease in the intermolecular distances, and second, low temperature, which diminishes the thermal activity of the molecules. It is suggested that in this way a new ordered submicroscopic protoplasmic structure is produced which possesses new properties e.g., high water permeability and high resistance to dehydration and mechanical deformations.

LITERATURE CITED

[1] H. M. Müller-Thurgau, Landw. Jahrb, 9, 133 (1880).

[2] H. M. Müller-Thurgau, Landw. Jahrb., 15, 453 (1886).

[3] H. Molisch, Untersuchungen uber das Erfrieren der Pflanzen (G. Fisher, 1897).

[4] B. Lidforss, Lunds Universitäts Arsskrift, N. F. Aft 2, 1 (1907).

[5] N. A. Maksimov, Izv. Lesnogo Inst., 25, 1 (1913).

[6] A. Akerman, Studien über den Kältetod und die Kälteresistenz der Pflanzen nebst Untersuchunger über die Winterfestigkeit des Weizens. Berlinska Boktryckeriet, 1 (1927).

[7] R. B. Harvey, J. Agric. Res., 15, 83 (1918).

[8] J. T. Rosa, Miss. Agr. Exptl Stat. Res. Bull, 48, 1 (1921).

[9] R. Newton, J. Agric. Sci., 14, 178 (1924).

[10] I. I. Tumanov, Trudy po Prikl. Bot. Genet. i Selektsii, 25, 69 (1931).

[11] A. U. Pisek and R. Schiessl, Ber. Naturwiss. med. Vereins Innsbruck 47, 33 (1947).

[12] I. I. Tumanov and O. A. Krasavtsev, Fiziol. Rastenii, 2, 230 (1955).

[13] A. Sakai, Low Temperat. Sci. Ser. B, 14, 17 (1956).

[14] I. I. Tumanov, Physiological Bases of Winter Hardiness in Plants (Sel'khozgiz, 1940) [In Russian].

[15] I. I. Tumanov, Fiziol. Rastenii, 2, 283 (1955).

[16] I. I. Tumanov and T. I. Trunova, Fiziol. Rastenii, 5, 112 (1958).

[17] I. I. Tumanov and T. I. Trunova, Fiziol. Rastenii, 4, 397 (1957).

[18] I. I. Tumanov, Vestn. Akad. Nauk SSSR, 10, 111 (1957).

[19] I. I. Tumanov, Priroda 1, 112 (1959).

[20] I. I. Tumanov, Izv. Akad. Nauk SSSR ser biol. 2, 265 (1959).

[21] O. A. Krasavtsev, Fiziol. Rastenii 4, 570 (1957).

[22] E. Asahina, Contrib. Inst. Low Temperat. Sci. 10, 83 (1956).

[23] W. Tranguillini and K. Holzer, Ber. Dtsch. bot. Ges. 71, 143 (1958).

[24] J. Levitt and G. W. Scarth, Canad. J. Res. Ser. C, 14, 267 (1936).

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THE FREE AMINO ACID CONTENT OF LEAVES AND ROOTS OF MAIZE AS RELATED TO NUTRITIONAL CONDITIONS

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It is well known that the deficiency or absence of various nutrient elements in the plant's environment leads to an alteration of its metabolic processes and its chemical composition. The level of mineral nutrients exerts an influence primarily on the synthesis of proteins and amino acids and on other processes of nitrogen metabolism [1-3].

A more detailed study of the patterns of nitrogen metabolism as they are affected by nutritional regime showed that synthesis and levels of proteins, amino acids, amides, and other nitrogen compounds in plants are strictly dependent on mineral levels. One of the authors of this study, and also other workers have shown that a deficiency of the most important mineral nutrients (nitrogen, potassium, and phosphorus) leads to a reduction in the rate of protein synthesis, and to an accumulation of free amino acids in plant tissues [3-7]. The perfection of chromatographic methods of determining amino acids, and also the utilization of isotopes, provide new opportunities to make a more searching study of the processes involved in nitrogen metabolism and particularly of amino acid levels as they depend on nutritional status [8].

In a number of experiments it was established that a deficiency of microelements, boron, copper, zinc, manganese, or iron, and also an absence of chlorine, was associated with a significant increase in the free amino acid content in tissues of various plants, while a molybdenum deficiency was associated with a decrease in the free amino acid content [9-14]. It was also shown that levels of individual amino acids were variously affected by nutritional conditions.

Recently, certain patterns of change in amino acid content, associated with various degrees of availability of the macroelements – nitrogen, phosphorus, and potassium – were found. It was shown that improvement of nitrogen availability results in a reduction of amino acid levels. A phosphorus deficiency induces marked disturbance of nitrogen metabolism and causes a reduction in the amino acid content of leaves and roots [17 - 19]; in some experiments, however, no reduction was observed in leaves [16]. A potassium deficiency resulted in an increase in amino acid levels, while an improved potassium nutrition resulted in somewhat lower amino acid levels [11, 15, 16]. The relation of amino acid content to mineral nutrition has not yet been fully clarified, however, and results are sometimes even contradictory. We have therefore attempted to study in greater detail the effect of nitrogen, phosphorus, and potassium availability on the free amino acid content of leaves and roots of maize.

METHODS

Experimental material employed was Voronezhskaya 76 maize, which was grown in water culture on Hellriegels nutrient solution (pH 6.0). Plants were set out in pots on May 24. Thirty days later the third, fourth, fifth, and sixth leaves, as well as the roots, were anlyzed for free amino acids. Some of the plants were then transferred from a complete nutrient medium to one lacking nitrogen, phosphorus, or potassium; these plants were grown under these conditions for a prolonged period.

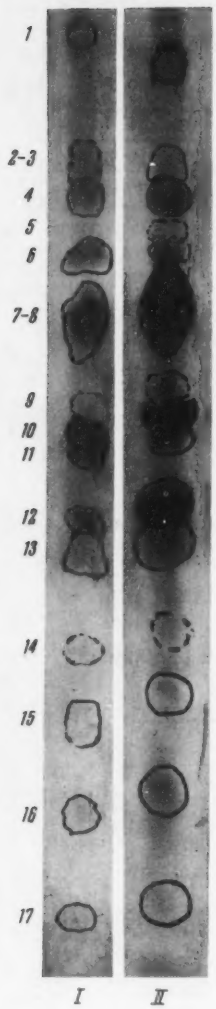

Fig. 1. Free amino acids in leaves and roots of 30-day-old maize plants, treatment NPK. I) Roots; II) leaves. Amount of extract spotted on the paper equivalent to dry weight of roots – 13.8 mg, of leaves – 21.0 mg. 1) Cystine; 2-3) lysine + histidine; 4) asparagine; 5) arginine; 6) glutamine; 7-8) aspartic acid + serine; 9) glycine; 10) glutamic acid; 11) threonine; 12) alanine; 13) amino acid X; 14) tyrosine; 15) γ-aminobutyric acid; 16) methionine + valine; 17) leucine.

The free amino acid content of leaves and roots was determined 3, 7, 17, and 27 days after the exclusion of nitrogen, and 3, 7, and 17 days after the exclusion of phosphorus and potassium. After a prolonged starvation (27 days), when the plants exhibited all the signs characteristic of nitrogen deficiency, they were supplied with one or four doses of nitrogen, based on Hellriegel's solution; the free amino acid content was determined 1, 4, 9, 24, 48, and 118 hours after the additions of nitrogen.

Amino acids were extracted from fresh leaves and roots with 80% alcohol until the extract no longer reacted with ninhydrin (7- to 8- fold extraction). The alcoholic extract was evaporated on a water bath and the residue dissolved in a weakly acidic medium; this was filtered and evaporated to dryness, and the amino acids were taken up in 1 ml of water acidified with HCl.

The free amino acid content was determined quantitatively by paper partition chromatography (chromatograph paper, Leningrad No. 2). The developing solution employed was n-butanol – acetic acid – water (4 : 1 : 1). To improve resolution of the acids, the solution was passed through the paper five times. In the determination of alanine, tyrosine and aminobutyric acid the chromatograms were developed for 20 hours longer, and for determination of amino acids with low Rf numbers the chromatograms were developed for 2-3 days in addition [15]. Amino acids were identified by the use of markers. After development the chromatograms were treated with 0.5% ninhydrin in acetone, and a day later with a solution of cupric nitrate in acetone.

For quantitative determination the spots were cut out and the pigment extracted with 7 ml of methyl alcohol for 1.5-2 hours. The concentration of pigment was determined in a colorimeter using a blue filter. Amounts of individual amino acids were calculated from calibration curves prepared for each amino acid.

RESULTS

Photographs of chromatograms which show the relative amounts of various amino acids occurring in alcoholic extracts from leaves and roots of 30-day-old plants which are supplied with all three of the macroelements under consideration (treatment NPK) are presented in Fig. 1. These chromatograms were made without running the developing solution completely through the paper so that the resolution of amino acids with low Rf's was not entirely satisfactory. It was shown that the following amino acids occur in both leaves and roots: cystine, lysine, histidine, asparagine, glutamine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, tyrosine, γ-aminobutyric acid, valine (and possibly methionine), leucine, and an unidentified amino acid with an Rf of about 0.4. Thus, no qualitative differences whatever were found in the amino acid composition of leaves and roots, but almost every amino acid occurred at a level two-four times lower in the roots than in the leaves (Table 1).

Upon determination of amino acid levels in 33-, 37- and 47-day-old plants, treatment NPK, (Table 1, Figs. 2 and 3) we found that they do not remain constant in the course of development but may undergo more or less marked changes. For example, the free amino acid content of leaves of 30-day-old plants was 5.27 mg per g dry weight, that of 33-day-old plants was 7.18 mg, that of 37-day-old plants was 12.24 mg, and that of 47-day-old plants had fallen abruptly to 2.11 mg. In roots, the free amino acid content had steadily risen from 30 to 47 days, and at 47 days it was twice as great as in the leaves.

TABLE 1

Changes with Age in Free Amino Acid Levels of the Leaves and Roots of Maize, Treatment NPK (in mg per g dry wt)

Amino acid	Leaves				Roots		
	plant age, days						
	30	33	37	47	30	37	47
Leucine	0.17	0.29	0.27	0.04	Traces	0.12	0.20
Valine + methionine	0.21	0.41	0.50	0.07	0.15	0.08	0.27
γ-Aminobutyric acid	0.26	0.25	0.16	Traces	0.18	0.20	0.06
Tyrosine	0.21	0.50	0.17	Same	0.17	Traces	0.01
Alanine	1.07	1.97	1.24	0.33	0.22	0.23	0.27
Threonine	0.25	0.00	0.11	0.05	0.10	Not det.	0.16
Glutamic acid	1.98	0.58	1.85	0.15	0.68	0.36	0.19
Glycine	0.00	1.53	0.25	Traces	Traces	0.11	0.05
Serine	1.16	0.00	2.52	0.49	0.22	0.79	0.69
Aspartic acid	Traces	0.20	3.86	0.40	Traces	0.05	1.00
Glutamine	Same	0.23	0.04	0.10	0.27	0.18	0.49
Arginine	»	0.54	0.05	Traces	Traces	0.19	0.11
Asparagine	»	0.45	0.24	Same	Same	0.24	0.30
Histidine + lysine	»	0.23	0.82	0.27	»	0.11	0.56
Cystine	»	Traces	0.16	0.21	»	0.25	Traces
Total amino acids	5.31	7.18	12.24	2.11	1.99	2.96	4.36

TABLE 2

Changes with Age in Free Amino Acid Levels of the Leaves and Roots of Maize Deprived of Nitrogen (in mg per g dry wt)

Amino acid	Leaves			Roots		
	without nitrogen, days					
	3	7	17	3	7	17
Leucine	0.15	0.18	Traces	0.13	0.11	0.00
Valine + methionine	0.21	Traces	Same	0.11	0.12	Traces
γ-Aminobutyric acid	0.17	0.15	0.00	0.17	0.14	0.08
Tyrosine	0.33	Traces	Traces	0.49	Traces	0.00
Alanine	1.12	0.22	0.11	0.32	0.31	0.13
Threonine	Traces	0.23	0.03	0.00	0.09	0.00
Glutamic acid	0.47	0.27	0.11	0.15	0.30	0.00
Glycine	0.49	0.09	0.00	0.25	0.00	Traces
Serine	0.11	0.09	0.10	0.00	0.22	0.02
Aspartic acid	Traces	0.22	Traces	0.30	0.00	Traces
Glutamine	0.17	0.13	0.03	0.37	0.25	0.52
Arginine	0.09	0.05	Traces	0.12	0.07	Traces
Asparagine	0.19	Traces	0.00	0.37	Traces	Same
Histidine + lysine	0.69	0.19	0.04	Traces	0.06	0.12
Cystine	Traces	0.12	Traces	Same	Traces	Traces
Total amino acids	4.19	1.94	0.42	2.78	1.67	0.87

During growth, the contents of alanine, aspartic acid, glutamic acid, glycine, serine, and γ-aminobutyric acid change most markedly, and the total amino acid content depends primarily on the levels of these compounds. In view of the fact that these amino acids are the ones most easily synthesized, either by direct amination of the corresponding carboxylic acids or by transamination reactions, it may be supposed that the free amino acid content in leaves and roots is determined on the one hand by the rate of entry of nitrogen into the plant and the existence of free ammonia reserves, and on the other hand by the rate of glycolysis and keto acid formation and the over-all rate of metabolism.

Because of the marked variations in amino acid content during plant development, it was necessary always to compare experimental treatments with the control treatment, in which plants had developed on a complete nutrient medium.

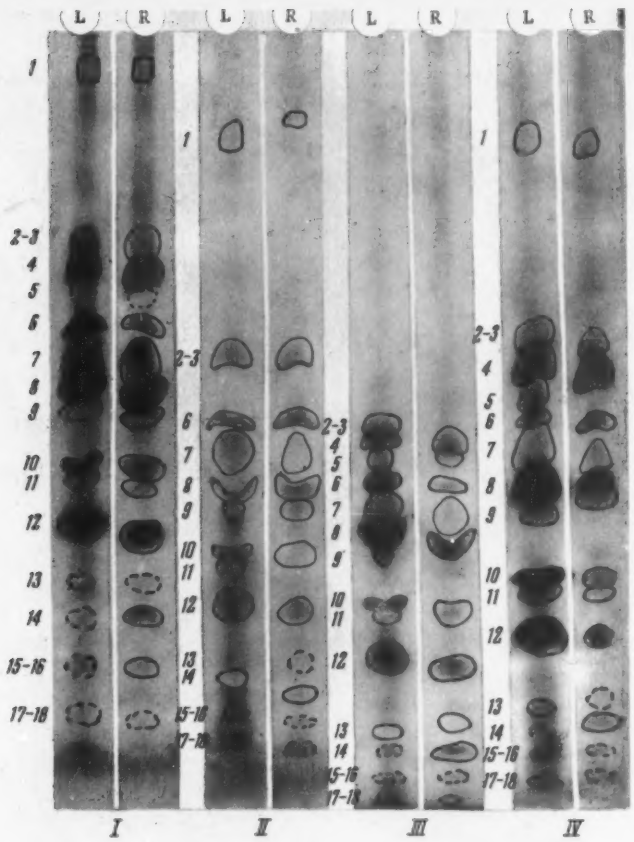

Fig. 2. Free amino acid levels in leaves and roots of maize seven days after exclusion of nitrogen, phosphorus, and potassium. I) Without potassium; II) without nitrogen; III) without phosphorus; IV) control NPK; L) leaves; R) roots. Dry weights: I, L) 11.5 mg; I, R) 6.7 mg; II, L) 12.1 mg; II, R) 8.9 mg; III, L) 12.9 mg; III, R) 8.9 mg; IV, L) 12.5 mg; IV, R) 9.5 mg. 1) Cystine; 2-3) lysine + histidine; 4) asparagine; 5) arginine; 6) glutamine; 7) aspartic acid; 8) serine; 9) glycine; 10) glutamic acid; 11) threonine; 12) alanine; 13) tyrosine; 14) γ-aminobutyric acid; 15-16) methionine + valine; 17-18) leucine + isoleucine.

Exclusion of nitrogen after a month's development on a complete nutrient medium resulted in a steady decline in the amino acid level of both leaves and roots. Chromatographic determinations showed that even one day after the removal of nitrogen there was a small reduction in the total amino acid content. The levels of asparagine, aspartic acid, glutamic acid, glycine, and alanine fell most markedly upon nitrogen deprivation, and the levels of other amino acids remained almost the same. Thus, a short-term nitrogen deficiency is manifested first in a reduction of the level of those amino acids which are formed by direct amination of organic acids, and the metabolism of other amino acids is almost unaffected.

Three days after the removal of nitrogen the total amino acid content was reduced almost twice as much as in control plants (treatment NPK); the levels of aspartic acid, asparagine, arginine, glycine, alanine, and γ-aminobutyric acid were reduced to the greatest extent. There was also a noticeable reduction in the levels of leucine, valine, and tyrosine (Table 2).

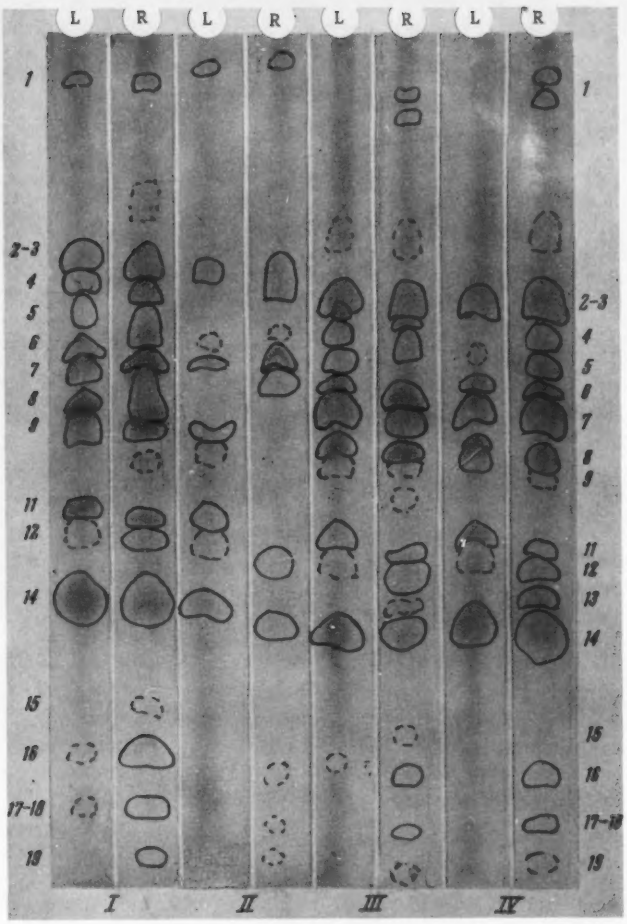

Fig. 3. Free amino acid levels in leaves and roots of maize 17 days after exclusion of nitrogen, phosphorus, and potassium. I) Control (NPK); II) without nitrogen; III) without potassium; IV) without phosphorus; L) leaves, R) roots. Dry weights: I, L) 15.1 mg; I, R) 9.5 mg; II, L) 16.0 mg; II, R) 9.7 mg; III, L) 17.7 mg; III, R) 9.3 mg; IV, L) 15.3 mg; IV, R) 9.7 mg. 1) Cystine; 2-3) lysine + histidine; 4) asparagine; 5) arginine; 6) glutamine; 7) aspartic acid; 8) serine; 9) glycine, 10) amino acid ×; 11) glutamic acid; 12) threonine; 13) amino acid ×; 14) alanine; 15) tyrosine; 16) γ-aminobutyric acid; 17-18) methionine + valine; 19) leucine.

After seven days, all amino acids, with the exception of cystine, threonine and leucine, had declined sharply in amount in both roots and leaves; after 17 days only insignificant amounts of the amino acids typical of maize were found (Table 2, Figs. 2 and 3).

With prolonged nitrogen starvation, not only were free amino acids depleted, but also other forms of nitrogen. It was shown that after 27 days' nitrogen starvation the total nitrogen content of the leaves had fallen from 49.9 to 8.8 mg per g dry weight, and that of the roots – from 34.6 to 9.9 mg per g. The protein nitrogen content fell from 34.7 to 8.5 mg per g in the leaves, and from 13.1 to 8.5 mg in the roots.

TABLE 3

Free Amino Acid Content in Maize Roots After Application of Nitrogen to Plants (in mg per g dry wt)

Amino acid	Hours after introduction of N					
	0	1	4	9	24	48
Leucine	0.00	0.00	Traces	Traces	Traces	Traces
Valine	0.00	0.00	Same	Same	Same	Same
γ-Aminobutyric acid	Traces	0.03	0.03	0.14	0.16	0.31
Tyrosine	0.00	0.00	0.00	Traces	Traces	Traces
Alanine	0.05	0.06	0.07	0.25	0.24	0.69
Threonine	0.00	0.00	0.00	Traces	Traces	Traces
Glutamic acid	0.00	0.00	0.00	Same	Same	Same
Glycine	0.00	Traces	Traces	»	0.01	0.04
Aspartic acid	0.00	0.00	0.00	»	Traces	Traces
Glutamine	0.00	0.00	Traces	0.66	0.43	0.29
Lysine + histidine	0.00	0.00	0.00	Traces	Traces	Traces

TABLE 4

Changes with Age in Free Amino Acid Levels of Leaves and Roots of Maize Deprived of Phosphorus (in mg per g dry wt)

Amino acid	Leaves			Roots		
	without phorphorus, days					
	3	7	17	3	7	17
Leucine	0.19	0.31	0.00	0.21	0.17	Traces
Valine	0.45	0.20	0.00	0.29	Traces	Same
γ-Aminobutyric acid	0.30	0.14	0.00	0.80	0.34	0.06
Tyrosine	0.37	0.11	0.00	0.66	0.27	0.00
Alanine	1.76	0.90	0.15	2.96	0.40	0.24
Threonine	0.00	0.27	0.06	0.00	Traces	0.18
Glutamic acid	0.93	1.37	Traces	0.48	0.57	0.06
Glycine	1.68	0.24	Same	0.94	Not det.	Traces
Serine	0.00	0.78	0.20	0.00	0.53	0.21
Aspartic acid	0.33	1.84	0.24	0.28	Not det.	0.90
Glutamine	0.33	0.36	0.02	0.40	0.09	0.07
Arginine	0.33	0.05	Traces	0.40	0.01	0.06
Asparagine	0.65	0.06	0.00	0.45	Traces	0.25
Histidine + lysine	0.41	0.55	0.14	0.85	0.15	0.26
Cystine	Traces	0.26	0.00	Traces	0.26	Traces
Total amino acids	7.73	7.44	0.81	8.72	2.79	2.29

In order to study the rate of amino acid synthesis, plants deprived of nitrogen for 27 days were transferred to a complete nutrient solution containing either one or four doses of nitrogen, and the amino acid content was determined over short-time intervals thereafter. Results obtained in this series of experiments are presented in Fig. 4 and Table 3.

From these data it is evident that as early as one hour after introduction nitrogen the level of alanine and γ-aminobutyric acid is increased in the roots. At four hours, a further increase in the level of these compounds had occurred and traces of glutamine and glycine had appeared; at nine hours it was evident there had been a further rapid synthesis of alanine, γ-aminobutyric acid, and glutamine, as well as certain other acids, and traces of tyrosine, threonine and the basic amino acids histidine and lysine had appeared. Thus, in spite of the rapid flow of amino acids from the roots to the leaves and the rapid utilization of amino acids in protein synthesis (which takes place under conditions of nitrogen deficiency), the formation of many amino acids, including basic and aromatic acids, took place.

After 24 and 48 hours, all the amino acids characteristic of maize were observable in significant amounts in the roots.

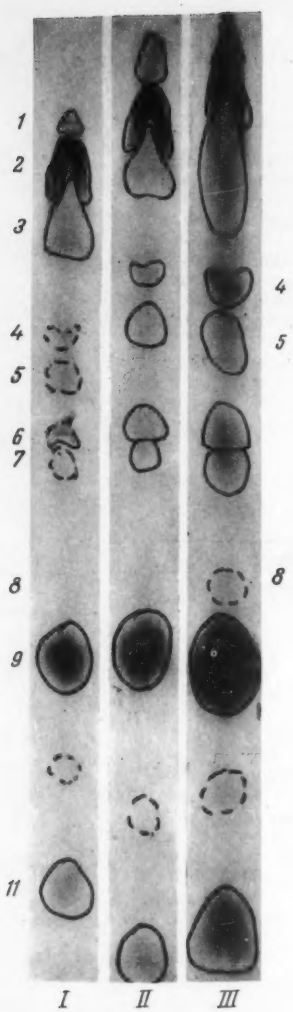

Fig. 4. Free amino acid content in roots of maize, treatment PK + 4N. I) Exposure; I) 9 hrs; II) 24 hrs; III) 48 hrs. Dry wt.: I) 18.7 mg; II) 26.8 mg; III) 24.3 mg. 1) Lysine + histidine; 2) glutamine; 3) aspartic acid; 4) glycine; 5) amino acid X; 6) glutamic acid; 7) threonine; 8) amino acid x; 9) alanine; 10) tyrosine; 11) γ-aminobutyric acid.

This experiment shows that maize roots are capable of synthesizing all the amino acids found in this plant in short periods; this lends additional support to the notion that the root system is an active synthesizing organ [17-23].

Determinations of amino acid content of leaves after introduction of nitrogen showed that during the first hours there was no noticeable accumulation of amino acids, and even after 24-48 hours the increase in amino acid content was very insignificant. Two days after the application of a four-fold dose of nitrogen, we found only traces of glutamine, aspartic acid, glycine, and glutamic acid in the leaves, but a significant amount of an unidentified compound ($R_f \sim 0.3$-0.4) giving a deep blue-violet color with ninhydrin. Under an ordinary nutritional regime this compound is not found in leaves. Only after 188 hours were traces of several compounds characteristic of maize found in the leaves. We are inclined to explain the fact that accumulation of amino acids is markedly slower in leaves than in roots in terms of a more rapid utilization of the amino acids formed in protein synthesis rather than in terms of a low rate of amino acid synthesis in the leaves.

Determining the rate of synthesis of amino acids in the roots and aerial organs of oats after addition of nitrogen to the nutrient medium, Turchin and co-workers [24] found that in roots alanine is accumulated after 30 minutes, glutamic acid – after 4 hours, asparagine – after 20 hours, and serine – after 44 hours; at 44 hours neither tryptophane nor histidine was detectable in the roots. In the aerial organs, only alanine was formed in the interval from 30 minutes to 4 hours after introduction of nitrogen; after 44 hours other amino acids were found. In general our data are in agreement with those of F. V. Turchin, but in all our experiments we found a more rapid synthesis of many amino acids in roots, including the basic and aromatic ones. We believe that basic and aromatic acids may be synthesized and accumulated even during the first 9 hours after introduction of nitrogen, instead of requiring a 30-40 hour period, as claimed by F. V. Turchin.

In the study of the effect of phosphorus on amino acid content, plants which had been grown 30 days on a complete nutrient medium were transferred to a medium lacking this element; analyses were performed at intervals from the 3rd to the 17th day after the transfer. Results of these analyses are presented in Table 4 and Fig. 2 and 3.

Three days after the removal of phosphorus from the nutrient medium, the amino acid content of the roots had increased markedly, and that of the leaves had not changed or had even increased somewhat. The increase in alanine, which is easily formed from pyruvic acid, was especially prominent in the roots. After seven days, the amino acid content of the leaves was somewhat reduced, while in the roots it had decreased threefold and was lower than in control plants. The levels of the most labile amino acids, alanine, glycine, arginine, asparagine and γ-aminobutyric acid, fell very sharply. With further phosphorus starvation, the amino acid level declined still more, especially in leaves, and on the 17th day there were only slight amounts of aspartic acid, alanine, serine, and certain other amino acids, the majority of the amino acids being almost completely absent.

TABLE 5

Changes with Age in Free Amino Acid Levels of the Leaves and Roots of Maize Deprived of Potassium (in mg per g dry wt)

Amino acid	Leaves			Roots		
	without potassium, days					
	3	7	17	3	7	17
Leucine	0.26	0.35	0.00	0.19	0.23	0.33
Valine	0.43	0.34	0.00	0.30	0.34	Traces
γ-Aminobutyric acid	0.33	0.12	Traces	0.67	0.25	0.07
Tyrosine	0.54	0.12	0.00	0.67	0.35	Not det.
Alanine	1.31	1.35	0.33	1.89	1.18	0.18
Threonine	0.00	0.25	0.10	0.00	0.06	0.09
Glutamic acid	0.45	0.53	0.25	0.80	0.87	0.04
Glycine	1.66	0.49	0.07	0.99	0.32	0.01
Serine	0.00	1.52	Traces	0.15	1.88	0.40
Aspartic acid	Traces	2.87	0.34	0.66	1.33	1.60
Glutamine	0.45	0.88	0.09	1.45	1.26	0.41
Arginine	0.40	0.24	0.00	0.54	0.44	0.41
Asparagine	0.52	0.51	Traces	1.20	1.40	0.31
Histidine + lysine	0.23	0.88	0.26	0.51	1.20	0.35
Cystine	Traces	0.34	Traces	—	0.73	—
Total amino acids	6.57	10.79	1.40	10.02	11.54	3.90

It is possible that, during the first days after removal of phosphorus, it is protein synthesis which is primarily affected, while at later periods amino acid synthesis is disturbed.

In our earlier experiments with kidney bean [15], it was found that with a phosphorus deficiency the amino acid content of the leaves was unchanged or even somewhat increased.

The absence of a decline in amino acid levels in these experiments should be explained in terms of a weak phosphorus starvation, but not specifically in terms of metabolic peculiarities in kidney bean. With a more severe starvation, the amino acid level is reduced even in the leaves (unpublished data of the author). It was shown by A. L. Kursanov [17] that phosphorus starvation in pumpkin results in reduced amounts of a number of amino acids and, in general, disturbances of nitrogen metabolism.

Results of determinations of amino acid content of leaves and roots 3, 7 and 17 days after removal of potassium from the nutrient medium are presented in Table 5 and Figs. 2 and 3. These data show that with weak potassium starvation (three and seven days) the amino acid content of the leaves and especially of the roots is markedly increased (cf. Tables 1 and 5). This increase was due chiefly to an increase in alanine, glycine, serine, aspartic acid, asparagine, and glutamine. It is apparent that a phosphorus deficiency primarily affects protein synthesis, and that the nitrogen which continues to enter the plant is incorporated chiefly into labile amino acids and amides. With a more severe starvation, such as occurs after 17 days, not only was protein synthesis disrupted, but also that of amino acids, and the total amino acid content was sharply reduced.

It should be pointed out that when nitrogen, phosphorus or potassium is excluded from the nutrient medium the free amino acid content of leaves declines more rapidly and to a greater extent than does that of roots. These facts may apparently be attributed not only to a high rate of synthesis in the root system, but also to a higher specific weight of the protein fraction in the leaves as compared with the roots.

Supplementary data on the effect of nutrition on the free amino acid content and the amino acid composition of plant proteins were obtained in experiments performed in 1958-1959 [25, 26].

SUMMARY

The dependence of the free amino acid content in leaves and roots of maize on nitrogen, phosphorus, and potassium nutrition was studied. The plants were cultivated during a month in water cultures containing a complete nutritional mixture and were then transferred to a medium in which nitrogen, phosphorus or potassium were separately excluded. The free amino acid content was determined 1, 3, 7 and 17 hours after change of the nutritional media.

Seventeen amino acids were found in the leaves and roots of maize cultivated in a complete nutritional medium. The free amino acid content did not remain constant during growth of the plant. The most sharp variations were found in aspartic and glutamic acids, alanine, serine, and glycine.

Twenty-four hours after exclusion of nitrogen the amino acid content was found to drop appreciably. Amino acids which are formed by direct amination of organic acids were the first to decrease. The amount of all other amino acids and proteins in the leaves and roots decreased after a sufficiently long period following exclusion of nitrogen.

After an hour in the presence of nitrogen, synthesis of alanine and aminobutyric acid was observed; after 4 hours glutamine and glycine were detected, and 9-24 hours after introduction of nitrogen all amino acids characteristic of maize were found to accumulate in the roots. Free amino acids accumulated much more slowly in the leaves.

Brief (3-7 days) exclusion of phosphorus and potassium from the nutritional medium either did not change or even somewhat enhanced the amount of free amino acids in the plants, but severe phosphorus or potassium deficiency led to a sharp decrease of the amino acid content.

LITERATURE CITED

[1] D. N. Pryanishnikov. The Role of Nitrogen in the Life of the Plant and in Agriculture in the USSR [in Russian] (Izd. AN SSSR 1945).

[2] A. Chibnall, Protein Metabolism in the Plant (London, 1939).

[3] D. A. Sabinin. Physiological Principles of Plant Nutrition [in Russian](Izd. AN SSSR 1955).

[4] Zh. A. Medvedev, Collected Papers, Plant Nutrition and Fertilizing [in Russian] (1954).

[5] A. G. Shestakov and B. P. Pleshkov, Doklady Akad. Nauk SSSR 98, 1, 149 (1954).

[6] A. G. Shestakov and B. P. Pleshkov, Izv. TSKhA 3, 139 (1955).

[7] B. P. Pleshkov, Fiziol. Rastenii 5, 2 (1958). •

[8] A. L. Kursanov, The Use of Labeled Atoms in the Development of the Scientific Foundations of Plant Nutrition [in Russian] (Izd. AN SSSR, 1954).

[9] R. A. Steinberg, J. D. Bowling and J. E. McMurtrey, Plant Physiol. 25, 2, 279 (1950).

[10] F. J. Richards and R. G. Coleman, Nature 170, 4324, 460 (1952).

[11] E. J. Hewitt, E. W. Jones and A. H. Williams, Nature 163, 4148, 681 (1949).

[12] J. W. Possingham, Austral. J. Biol. Sci. 19, 4, 534 (1956).

[13] Noboru Shimose and A. Mifune, J. Sci. Soil and Manure (Japan) 27, 6, 215 (1956).

[14] A. A. Sobachkin, Izv. TSKhA 3, 55 (1958).

[15] B. P. Pleshkov, Sh. Ivanko and G. V. Antonova, Doklady Akad. Nauk SSSR 117, 6, 1070 (1957). •

[16] V. P. Dadykin and Z. S. Igumnova, Fiziol. Rastenii 3, 431 (1956).

[17] O. N. Kulaeva, E. I. Silina and A. L. Kursanov, Fiziol. Rastenii 4, 520 (1957). •

[18] A. L. Kursanov, Izv. Akad. Nauk SSSR, ser. biol. 6, 689 (1957).

[19] A. L. Kursanov, O. F. Tueva and A. E. Vereshchagin, Fiziol. Rastenii 1, 12 (1954).

[20] A. L. Kursanov, Voprosy Bot. 1, 131 (1954).

[21] A. L. Kursanov and O. N. Kulaeva, Fiziol. Rastenii 4, 322 (1957). •

• See English translation.

[22] N. A. Pristupa and A. L. Kursanov, Fiziol. Rastenii 4, 417 (1957). •

[23] V. P. Dadykin and Z. S. Igumnova, Fiziol. Rastenii 3, 259 (1956).

[24] F. V. Turchin, M. A. Guminskaya and E. G. Plyschevskaya, Collected Papers; Labeled Atoms in Investigations of Plant Nutrition and of the Use of Fertilizer (Izd. AN SSSR, 1955) [in Russian].

[25] B. P. Pleshkov and L. Fowden, Izv. TSKhA, 5 (1959).

[26] B. P. Pleshkov and L. Fowden, Nature 183, 1445 (1959).

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• See English translation.

THE RATE AND QUALITATIVE TREND OF PHOTOSYNTHESIS IN CHLOROTIC APPLE TREES

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In the Chuisk valley, as in other regions of Kirgizia, many fruit orchards frequently become chlorotic. As a result of this their productivity severely declines, and when chlorosis is excessively severe they die.

Our investigations have shown that the basic cause here for this orchard ill is the periodic rise of a water table having a clearly alkaline reaction which reaches pH 8.3 and, in conjunction, the conversion of iron into a form insoluble or only slightly available for plant use. Threeto five days after the chlorotic apples have been sprayed with a dilute solution of an iron salt the leaves usually begin to green.

In order to devise a scientifically founded measure for combatting this ill with the objective of increasing orchard productivity, the need arose to study the physiological peculiarities of chlorotic plants as they related to the specific edaphoclimatic conditions of the region. In this regard the study of the rate and qualitative direction of the photosynthesis process is especially interesting.

So far as healthy green plants are concerned there is already much data in the literature which elucidates to an appreciable degree these exceedingly complex problems of plant physiology.

It has been demonstrated that in the leaves of healthy green plants not only carbohydrates originate in the process of photosynthesis but also other organic substances, among them proteins, and that the qualititative orientation of this process is determined both by hereditary and the external conditions, and also by the physiological condition of the plant itself [1-5 etc.].

Judging from data in the literature the question concerning the qualitative trend of the process of photosynthesis in chlorotic plants remains as yet uninvestigated.

Minasyan [6] found that acidity was greater and the amount of soluble-sugar was less in chlorotic leaves of the cultivated sunflower. However, according to the data of Ivanov and Vasil'eva [7], the amount of carbohydrates, reduced materials, and ascorbic acid, is greater in the leaves of chlorotic apple trees. However, such changes may be caused not only by the trend of photosynthesis but also by the further metabolism of the original assimilates.

The question concerning the dependence of photosynthesis upon the degree of chlorosis, particularly in apple trees has been little investigated.

For these reasons, the Department of Plant Physiology of Kirgiz State University in 1953-1956 undertook a special investigation oriented toward the study of the physiology of chlorosis in the apple tree. In 1955, in addition to a number of other physiological indicators, the process of photosynthesis, its rate, and qualitative trend, was studied in chlorotic apples.

The experiments were conducted in typical chlorotic orchards of the Frunze Sovkhoz of the Kyzyl-Askersk district which is located in a zone where the ground water subsides in summer.

TABLE 1

Rate of Photosynthesis and Chlorophyll Content in Separate Halves of the Same Leaf

Sample No.	Leaf-halves	Chlorophyll, mg/g dry wt.	Photosynthesis, cpm	Ratio chlorotic to greening		Experimental conditions			
				chlorophyll	cpm	exposure begun	duration min	temperature, °C	illumination, Lux
1	Chlorotic	0.66	269	1.41	3.15	15^{35}	7.0	23.0	41 300
	Greening	0.93	847	—	—	—	—	—	—
2	Chlorotic	0.71	696	1.37	2.19	16^{10}	8.5	25.5	30 980
	Greening	0.97	1523	—	—	—	—	—	—
3	Chlorotic	0.58	1305	1.67	2.06	16^{35}	8.0	26.0	39 600
	Greening	0.97	2689	—	—	—	—	—	—
4	Chlorotic	0.71	545	1.68	1.22	17^{00}	8.0	23.5	7600
	Greening	1.19	664	—	—	—	—	—	—
5	Chlorotic	0.58	867	1.67	2.81	17^{30}	6.0	21.5	13 830
	Greening	0.97	2438	—	—	—	—	—	—
6	Chlorotic	0.58	123	1.91	2.14	18^{00}	6.5	20.0	2160
	Greening	1.11	264	—	—	—	—	—	—
7	Chlorotic	0.84	332	2.0	2.26	18^{50}	6.5	19.0	3650
	Greening	1.68	752	—	—	—	—	—	—

METHODS

We used C^{14} to study the rate and qualitative trend of photosynthesis in green and chlorotic leaves. We caused the chlorotic leaves to green-up by spraying them with a solution of iron, or injecting it into the plant.

For each exposure we selected leaves of the same age and from the same position on the shoot. Just before the experiment began the leaves were severed from the shoot and, without delay, placed in water-filled slots in rubber stoppers inside a chamber which was then hermetically sealed. With special apparatus a gas mixture containing $C^{14}O_2$ was drawn through the chamber at a rate of 100 liters per hour.

Healthy and chlorotic leaves and leaves just commencing to green-up were placed in the chamber together in order to provide uniform conditions for C^{14} assimilation.

TABLE 2

Qualitative Trend of Photosynthesis in Chlorotic Apple Leaves in Relation to Their Treatment

Fraction	Extracted substances, mg			Activity by fraction					
				cpm			% of total sample activity		
	chlorotic leaves	greening-up		chlorotic leaves	greening-up		chlorotic leaves	greening-up	
		by spraying	by injection		by spraying	by injection		by spraying	by injection
Benzene	43	93	102	383	1330	1418	4.9	8.0	5.9
Alcohol	194	235	275	5102	11 374	17 518	65.9	68.0	73.3
Aqueous	191	122	99	1260	2147	2426	16.3	12.9	10.2
Acid	277	258	263	748	1445	1973	9.7	8.7	8.3
Alkali	168	178	164	202	338	459	2.6	2.0	1.9
Wet cellulose	127	114	97	51	80	97	0.7	0.5	0.4
Total activity of sample	—	—	—	7746	16 714	23 891	100	100	100

Note: Total weight of analyzed sample, 1000 mg.

Warburg buffer seven served as the source of carbon dioxide. Two liters of this buffer contained 1.32 microcuries of C^{14}. After a 7-15 minute exposure the leaves were removed from the chamber and killed in boiling alcohol. Since part of the substances was extracted by the alcohol, each leaf was fixed in a separate, short, wide-mouthed flask, and after fixation the alcohol with the substances dissolved in it were collected on a filter paper. The samples were dried to constant weight at 70° before analysis. The substances adsorbed by the paper were extracted with hot alcohol and the alcohol was evaporated. The residue left after evaporation of the alcohol and the fixed leaves themselves were carefully triturated to a very fine powder in a porcelain dish. Using an end-counter, T-25-BFL, adjusted for beta radiation, we determined the total C^{14} radioactivity of this powder according to directions in the literature [8].

In order to investigate the qualitative trend of the photosynthesis process in chlorotic leaves, we used an average sample of one or two grams of powder from all exposures within each experimental treatment: 1) chlorotic leaves, 2) leaves greening-up after spraying with a solution of iron and, 3) leaves greening-up after the injection of iron tannate into the roots.

Each of the average samples was successively fractionated using the method proposed by Zalenskii et al. [9, 10].

The pigment content of the apple leaves was determined by the method of Godnev and Terent'ev [11].

EXPERIMENTAL RESULTS

Results obtained from one of the experiments in which we determined the rate of photosynthesis in even-aged apple leaves using C^{14} and which discriminates between the different amounts of chlorophyll associated with chlorosis are shown in the following table:

Experimental indicator	Determinations on separate samples, cpm					
Leaf chlorophyll, mg/dm^2	4.63	3.53	2.38	1.75	1.14	0.52
Photosynthesis rate, cpm	1828	1477	1193	1007	715	216
Assimilation number	395	418	501	575	627	416

It is apparent from the data that the rate of photosynthesis decreases when the amount of leaf chlorophyll decreases. It can be seen from these data that the indicative assimilation number does not decrease with a decrease in chlorophyll content but steadily increases. Only in the case where the amount of chlorophyll falls below 1 mg/dm^2 of leaf area in yellowish leaves becoming chlorotic does the assimilation number decrease very appreciably. Nevertheless, even with this chlorophyll content, the index remains larger than it is in the group of leaves containing a much larger amount of chlorophyll (4.63 mg/dm^2). This shows that weakly chlorotic apple leaves are able to fully exploit the potential of chlorophyll to synthesize and in this respect they are analogs of the light green leaves of healthy plants.

The disparities between the rate of photosynthesis and the assimilation number (cpm/mg chlorophyll) and the change in the chlorophyll content of chlorotic apple leaves may indicate that the rate of photosynthesis depends upon not only the amount of chlorophyll but also upon the over-all physiological condition of the assimilating cells. The latter is greatly worsened in chlorotic leaves when their chlorophyll content falls to less than 1 mg/dm^2 of leaf surface.

In Table 1 are shown data from determinations of the rate of photosynthesis and the amount of chlorophyll in individual halves of one and the same leaf in relation to chlorophyll content, and the degree of chlorosis of the leaf half.

The difference between the chlorophyll content of the two halves of chlorotic leaves was achieved by daubing one of the halves of a large leaf with a 0.5% solution of iron sulfate heptahydrate. The leaves were treated July 7, 1955 and the physiological indices were assessed on July 20. Apple variety Papirovka was used as an experimental plant.

From the data in Table 1 it follows that in all cases in this experiment the rate of photosynthesis was greater in the experimental halves of chlorotic leaves (those halves which were greening-up) than in the controls. The

TABLE 3

Chlorophyll and Carotinoid Content of Apple Leaves Chlorotic in Differing Degrees

Degree of chlorosis	mg/g leaf dry weight		Total pigments	Carotinoids % of total pigments	Chlorophyll Carotinoids
	chlorophyll	carotinoids			
0	5.66	2.12	7.78	27.2	2.67
1	4.52	1.83	6.35	28.8	2.47
2	3.47	1.60	5.07	31.5	2.10
3	2.48	1.39	3.87	35.9	1.78
4	1.48	0.96	2.44	39.3	1.54
5	0.67	0.66	1.33	49.6	1.02

photosynthesis rate in almost all cases exceeded the corresponding difference in chlorophyll content. This latter fact may also indicate that not only the amount of chlorophyll but also the physiological condition of the assimilating cells of even separate parts of the leaf, the leaf-half in the case given, exerts an influence upon the rate of photosynthesis. As our experiments show, not only does the chlorophyll content of the leaf-half treated with the solution of an iron salt change exceedingly quickly but so also do other physiological indicators. None of these leaf-halves could be distinguished from green, healthy leaves by our physiological indicator.

A trial of different leaves showed that the injection of an iron salt directly into the bole or root of affected trees is the most effective method for treating apple chlorosis.

In a number of cases the greened-up leaves of injected trees contained more chlorophyll than the leaves of trees externally healthy to the eye. For example, a check of the chlorophyll content of chlorotic Aport apple leaves and their analogs on the basis of growth and condition showed on July 31, 1955, that healthy leaves and those which greened-up after they were sprayed with solutions of ferric chloride or after the injection of iron sulfate could be typified in this way: chlorotic leaves contained 0.19 mg of chlorophyll per gram of dry weight, healthy leaves 1.25 mg, leaves greened-up by sprinkling 1.85 mg, and leaves greened-up by injection of iron sulfate, 3.03 mg.

This indicates that in the orchards of Frunze Sovkhoz and in outwardly normal leaves, i.e., in normally green apple leaves, we actually find initial stages of chlorosis which, however, in these healthy leaves do not progress far.

The results obtained from studying changes in the qualitative trend of the photosynthetic process in chlorotic apple leaves in relation to their treatment are presented in Table 2.

From the data for all fractions shown in Table 2, it is apparent that the photosynthesis rate of chlorotic apple leaves is definitely less than in leaves turning green. Regardless of the degree of leaf chlorosis the alcohol fraction has the largest amount of activity, particularly in leaves greened-up by injection of the roots with iron tannate. In this case the indicator in the greened-up leaves is 3.1 times larger than the average of all fractions, while in the leaves greened-up with solutions of iron salts it is only 2.16 times greater. This fact may indicate that under our conditions the injection of roots with iron tannate is a more effective method for combating chlorosis than spraying the leaves with solutions of iron-containing salts.

The aqueous and acid fractions are also distinctive in their indications of relatively high activity. The others, particularly the wet cellulose fraction, are characterized by a very low activity.

The Table 2 data on the fractions is of particular interest in relation to questions concerning the qualitative trend of photosynthesis in chlorotic plants.

In examining the data on the amount of extracted material and the activities (cpm) of the separate fractions of chlorotic and greened-up leaves it is easy to see that there is relatively more material in the aqueous and acid fractions of the chlorotic leaves, and that the contents of the alkali fraction of chlorotic and greening-up leaves differ little.

The data of Table 2 permit us to assume that one physiological feature of chlorotic apple leaves is not only the generally lower rate of photosynthesis, but also the change in its qualitative trend in the direction of a decreased synthesis of water- and alcohol-soluble substances among the foremost of which are the soluble sugars, pigments, and other organic substances, and also a trend toward a relatively augmented synthesis of water-, acid-, and alkali-soluble substances (predominantly organic acids, amino acids, and proteins) and an increase in the wet cellulose fraction.

A change in the qualitative trend of the process of photosynthesis in chlorotic leaves can be in part explained by an alteration of their pigment system in the direction of a reduced chlorophyll/carotinoid ratio. The

data in Table 3, in which are shown the results of a determination of chlorophyll and carotinoids in relation to differing degrees of chlorosis in the leaves of apple variety Papirovok gives an idea of the kind of changes which occur in the pigment composition of apple leaves in relation to their degree of chlorosis.

From the data of Table 3 it is apparent that the greater the degree of chlorosis, the relatively greater is the carotinoid content of the leaves. For example, there is nearly as much chlorophyll as carotinoids in very severely chlorotic leaves (group 5) while the chlorophyll content of dark green leaves (group 0) is 2.67 times that of carotinoids.

The fact that chlorotic leaves are distinguished from green ones by not only a very low chlorophyll content, but also by a relatively large carotinoid content (in relation to chlorophyll) indicates that yellowed, chlorotic leaves should absorb relatively more blue and very little red light which inevitably should affect the kind of products from photosynthesis.

Nichiporovich [4] considers that carotinoids can absorb up to 30% of the total solar energy absorbed by the leaf. The carotinoids absorb most completely the blue wavelengths, a quantum of which possesses about twice the energy of a quantum of red light, and, therefore, may be highly involved in energy aspects of the process of photosynthesis.

Through his experiments with raising plants in red and blue light Voskresenskaya [2] showed that carbohydrate formation in plants is stimulated by red light and formation of proteins and organic acids by blue light.

It is evident that the change in the qualitative trend of the photosynthesis process in chlorotic leaves compared to green ones can be explained by a qualitative change in their pigment system which conjunctively alters the spectral composition of the solar energy absorbed by the two kinds of leaves: Our other experiments showed that, in addition to the peculiarities mentioned, a greened-up leaf contained more water and more ash elements and furthermore, transpired much more weakly than a chlorotic leaf, which of course is not a cause but a consequence of its chlorosis.

In conclusion, it should be emphasized that when apple leaves beginning to turn chlorotic are sprayed with solutions containing iron (and even more so when afflicted trees are injected with an iron solution) an exceedingly great change in the physiology of the entire tree occurs which in many respects causes them to resemble trees having green leaves without visible signs of chlorosis.

At the same time, the pigment content, especially chlorophyll, is abruptly increased, the leaves turn green, the water-soluble sugar content increases, the water and ash content decreases, the ability to regulate stomata movement increases together with a rise in the rate of transpiration, and the process of photosynthesis speeds up. As a result the plants in terms of physiological indications resemble plants with normal, green, outwardly healthy leaves.

SUMMARY

1. A very important cause of apple chlorosis in the Chuisk valley is a deficiency in iron physiologically available to the plant arising from conversion of the soil iron into an insoluble form because of the high pH of the soil solution.

2. A deficiency of physiologically available iron in the apple tree, especially during a period of rapid growth, primarily retards pigment synthesis and alters the ratio of chlorophyll to carotinoids, since with increasing chlorosis the ratio of carotinoid to chlorophyll in the leaf increases, and the leaf turns yellow.

3. Such a change in the green and yellow pigments of chlorotic leaves doubtlessly affects the spectral composition of the light absorbed by these leaves, and leads to a change in not only the ratio but also the qualitative direction of the photosynthesis process.

4. Chlorotic leaves differ by having a much lower rate of photosynthesis than healthy leaves. Chlorotic apple leaves synthesize relatively more materials of the aqueous-, acid-, and alkali-soluble and wet cellulose fractions, and less material of the alcohol and benzen fractions than do healthy plants.

5. The introduction of iron compounds into apple leaves boosts their pigment content, especially their chlorophyll content, and leads to a substantial increase in the rate of photosynthesis. Such greened-up leaves according to other physiological indications also resemble green leaves (without external signs of chlorosis).

LITERATURE CITED

[1] V. A. Brilliant, Photosynthesis as a Vital Process in Plants (Izd. AN SSSR, 1949). •

[2] N. P. Voskresenskaya, Doklady Akad. Nauk SSSR 72, 1, 173 (1950).

[3] A. A. Nichiporovich, Inst-a Fiziol. Rast. im. K. A. Timiryazeva, AN SSSR VIII, No. 1 (1953).

[4] A. A. Nichiporovich, Light and the Carbohydrate Metabolism of Plants (Photosynthesis) (Izd. AN SSSR, Nauchno-Populyarn. Seriya, (1955). •

[5] V. V. Sapozhnikov, Proteins and Carbohydrates of Green Leaves as Products of Assimilation (Tomsk, 1894). •

[6] S. M. Minsayan, Izv. AN. Arm SSSR, Biol. i Sel'sko-Khoz. Nauk, VIII, 63 (1955).

[7] S. M. Ivanov and L. A. Vasil'eva, Izv. Mold. Filiala AN SSSR, No. 6 (26), 65 (1955).

[8] V. Bochkarev, Kerim-Markus, M. L'vova and Ya. Pruslin, Measurement of the Activity of Beta- and Gamma Emitters (Izd. AN SSSR, 1953). •

[9] O. V. Zalenskii, O. A. Semikhatova and V. L. Voznesenskii, Methods Employing Radio-Carbon C^{14} for the Study of Photosynthesis (Izd. AN SSSR, 1955). •

[10] O. V. Zalenskii, V. L. Voznesenskii, M. M. Ponomareva and T. P. Shtan'ko, Bot. Zhurn. 40, 348 (1955).

[11] T. N. Gonev and M. V. Terent'ev, Trudy Inst-a Fiziol. Rast. im. K. A. Timiryazeva, AN SSSR, VII, No. 1 (1950).

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METABOLISM OF PUMPKIN ROOTS DURING SOIL DROUGHT

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In a previous work [1] it has been shown that as the soil dries out the leaf content of phosphorylated compounds in a number of mesophytes decreases coincidentally with an accumulation of simple sugars. Apparently, the correlation between simple sugars and phosphorylated compounds is an expression of a slowing-down of the carbohydrate-phosphorus metabolism and has been linked with disruption of the normal pathways through which respiratory energy is utilized.

In order to study features of the energy metabolism in relation to plant water-supply conditions, we decided to follow the conversion of sugars in the roots, particularly since data at hand indicates that root absorption and synthesis activity is reduced during a water deficit [2, 3]. Although the study of root metabolism during drought is, of course, of interest in itself, we turned to the root system primarily because here, in contrast to the leaf, it is possible to show perfectly the influence of photosynthetic phosphorylation upon the balance of the compounds which interest us.

In summarizing all that has been done in the study of the circulation of organic substances in the plant and the role of the root system in this circulation [4-14] we must mention the following conditions which have considerable significance in all further discussion. Root system vitality, its growth, absorption and synthesis functions, depend to a very large degree upon the influx and the subsequent aerobic transformation of assimilates, the bulk of which consist of simple sugars. All of the diversity of substances required for the growth and functioning of the root itself and a number of the compounds formed which are necessary for growth and development of the aerial organs, certain of these compounds being exceedingly specific, originate basically from the conversion of sugars.

The sugars are rapidly transformed through glycolysis and the Krebs cycle into organic acids which serve as acceptors for mineral nitrogen compounds absorbed from the soil. In this way alanine, glutamic acid, and aspartic acid are synthesized and from these keto acids many other amino acids detected in the roots and sap are formed by transamination. Amides are also synthesized. A portion of the amino acids and amides formed and also a portion of the organic acids are translocated as constituents of the sap into the aerial organs where they are involved in a number of further conversions. The remainin fraction of these compounds is used in the root system itself, primarily in growth, and is incorporated during the process of fundamental protein synthesis.

For experimental purposes we selected the pumpkin in which the circulation of organic materials and the role of the root system in this circulation has been investigated most fully so far [4-6, 10, 12, 14-18]. Plants of the variety Mozoleevskaya were raised in metal Wagner vessels holding 5.5 kg of over-dry soil, in the greenhouse during 1957-1958. About the time that two true leaves appeared, the soil in all of the vessels was moistened to 60% of field capacity. The plants were then separated into two groups. Plants of the first group serving as controls were kept the entire time under conditions of optimal moisture supply (70-80% of field capacity •). The second group was subjected to gradually increasing soil moisture tension. The soil moisture slowly decreased such that after 40-50 days it was 10-15% of F.C. After this moisture level was reached the experimental vessels were irrigated and the soil moisture was rapidly brought up to 70% of F.C. At the end of the experimentthe plants were about 70 days old.

• Subsequently abbreviated as F.C.

Fig. 1. Pumpkin plants during the initial period (A) and at the end of drought (B) Left) control plant; right) soil drought.

TABLE 1

Pumpkin Plant Characteristics at the Very End of the Drought Period

Year	Age, days	Days elapsed	Treatment	Roots, dry wt/ plant		Shoot, dry wt/ plant		No. of leaves	
				mg	relative	g · 10	relative	total	living
1957	66	49	Control	980	100	560	100	27	26
			Drought	330	33.7	25	4.4	13	5
1958	59	49	Control	743	100	228	100	24	22
			Drought	148	19.9	17	7 4	10	3

Fertilizer in the form of NH_4NO_3, KH_2PO_4, and KCl were added when the vessels were filled with soil (greenhouse soil with one-tenth part of river sand added). In addition, during the vegetative period split fertilizer applications of the same salts and also $FeCl_3$ were made. It should be mentioned that when the experiment was set up the matching of an appropriate nutrient regimen with different soil moisture regimes presented certain recognized difficulties since the nature of drought effects depends to a great degree upon the conditions of mineral nutrition [19]. In aiming to a certain extent to achieve a matching between the amounts of irrigation water and mineral fertilizers, we added less fertilizer to the drought treatment (one third in 1957, one fifth in 1958).

Samples were taken both during the drought period beginning at the time when a rather noticeable lag in growth was detected and also when the drought was terminated and the plants were reirrigated. In 1957 the roots were killed with steam and then dried at 50°. In 1958 the roots were killed with liquid nitrogen and vacuum freeze-dried. The entire root system of the plant was killed. Although in this way functionally different parts of the root system were mixed, we considered all of such samples taken in a given case as occurring naturally, since we are aware of the appreciable differences in the growth and structure of the roots of the plants in both treatments and the differences in sampling conditions. Any endeavor to differentially analyze various zones of the root system doubtless would lead to serious errors because of the extraordinary difficulty in sampling comparable material. In addition to the roots themselves, we analyzed the sap for characteristic products of vital activities which are

TABLE 2

Total and Specific Contents of Mineral Phosphorus and Acid-Soluble Phosphoorganic Compounds in Roots toward the End of Drought (μgP) 1957

Treatment	P/g dry wt		Total P in root system		Mineral P/ Acid-soluble organic P
	Mineral	Acid-soluble organic	Mineral	Acid-soluble organic	
Control	1971	1541	1932	1510	1.28
Drought	2957	843	976	278	3.51

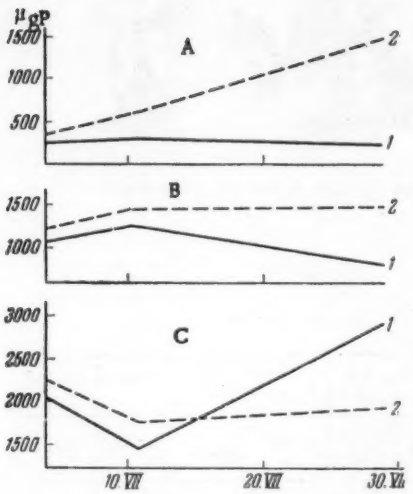

Fig. 2. Total (A) and specific content (B) of acid-soluble phosphoorganic compounds and the amount of inorganic phosphorus (C) in pumpkin roots. 1) During soil drought 2) Control plants. 1957.

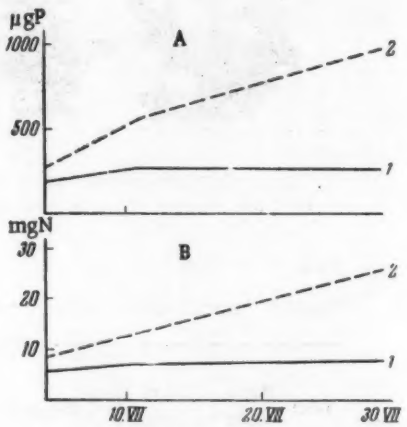

Fig. 3. Total content of ribonucleic acid (A) and protein nitrogen (B) in pumpkin roots. 1) During soil drought 2) Control plants. 1957.

supplied to the aerial organs by the roots and we also analyzed the leaves from various storeys with the object of more fully investigating the reciprocity of physiological processes in organs of the shoot and the root system. Sap was collected for twenty-four hours from stems cut off during the morning hours.

In the roots we determined sugars, phosphoorganic compounds of the acid-soluble fraction, inorganic phosphorus, nucleic acids, organic acids, amino acids, amides and total protein and nitrate nitrogen. In the sap we determined sugars, amino acids, amides, organic acids (exclusive of keto acids), total and inorganic phosphorus, and total, nitrate, ammonia, and amide nitrogen. The sap was analyzed without preliminary treatment. In the leaves we determined total and protein nitrogen, sugars, acid-soluble phosphoorganic compounds, and inorganic phosphorus.

We ascertained the weight of the root system and aerial organs, the amount of sap in a single plant, and the number of leaf storeys.

We determined the sugars of both water and alcohol extracts chromatographically using the solvents and developers recommended by Boyarkin [20]. Acid-soluble compounds were separated by extracting the material three times with 5% cold trichloroacetic acid. Further fractionations were made according to the technique of Meshkova and Aleksakhina [21]. Phosphorus was determined according to Fiske - Subbarow. Various solvents were

TABLE 3

Specific and Total Content of Pyruvic and α-Ketoglutaric Acids in Pumpkin Roots at the End of Drought (μgP) 1958

Treatment	Pyruvic acid		α-Ketoglutaric acid	
	per g dry wt.	whole root system	per g dry wt.	whole root system
Control	2790	2074	477	354
Drought	4750	704	412	61

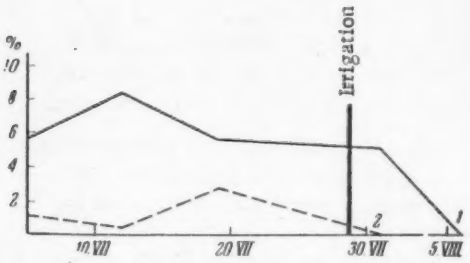

Fig. 4. Amide nitrogen in pumpkin sap (% of total nitrogen including nitrate). 1) During drought. 2) Control plants. 1957.

used in chromatographing the phosphorus compounds [22]. The following solvent was also used for investigating ATP and other nucleophosphates: propanol - 25% ammonia - water (6 : 3 : 1) with the paper preliminarily rinsed with 0.5% versene. Nucleic acids were determined according to Smith and Thannhauser. DNA was precipitated according to Abelev [23].

Keto acids were extracted with 6% perchloric acid and converted to hydrazones [14]. Chromatographic separation was made with the solvents: 1) n-butanol-ethanol-phosphate buffer (pH 7) (4 : 5 : 1) and 2) n-butanol-ethanol-buffer (4 : 1 : 5), the spots eluted with ethanol and the solution analyzed colorimetrically. The other organic acids were extracted with acidified ethyl ether. They were partitioned on paper using the following solutions: 1) n-butanol-formic acid-water (18 : 2 : 9) and 2) ethyl ether-formic acid-water (14 : 0.2 : 1.8) or (14 : 1 : 1). They were developed with brom-phenol blue. In addition, in the interest of better identification, we examined the flourescence of the spots under ultraviolet light after developing the chromatogram with a solution of ammoniated $AgNO_3$.

Amino acids and amides extracted with 70% ethanol were determined using various solvents and developers [24]. Total nitrogen was determined by the micro-Kjeldahl method, protein was precipitated according to Barnshtein. Nonprotein nitrogen was assessed by the difference between total and protein nitrogen. Nitrate was determined by reaction with disulfonic acid and measured colorimetrically. Ammonium and amide nitrogen in the sap were determined in the same way as in the work of Kulaeva [14].

Before presenting the results obtained we should pause at the methods employed to calculate the analytical data inasmuch as the basis for the results greatly depends upon the method of calculation itself. Calculations were usually made on the basis of a unit weight of tissue. However, as Sabinin has pointed out [4] and as has been decisively shown in his laboratory [25], in a determination of the absolute quantity of a substance the calculation must be made not upon the basis of a unit weight of tissue but upon the basis of a structural unit of the organism—an organ, a cell. The specific quantity of a substance, assessed on a unit weight basis, in reality, is an indicator of concentration which characterizes not the absolute quantity but the quantity of the given compound available, in a tissue. This quantity of material is thus, in fact, analogous to sap concentration. Therefore, in the same way as in calculating the quantity of materials exuded with the sap, the index of concentration must be multiplied by the volume of sap, since in all calculations of the concentration of a material in a root, the index of its content ought to be multiplied by the weight of the whole root system. We calculated the data both on the basis of the entire root system and also on the per-gram dry weight basis. In a number of cases we used still another method of calculation. In the future wherever a total content is discussed the absolute amount with respect to the entire root system will be intended. By specific content we mean the relative value which characterizes the concentration of a given compound per gram of dry weight.

For chromatographic determination of the total content of a substance the portion of the extract (or sap) placed on the chromatogram was proportional to the weight of the root system (or the sap respectively) in the plants of the treatment involved.

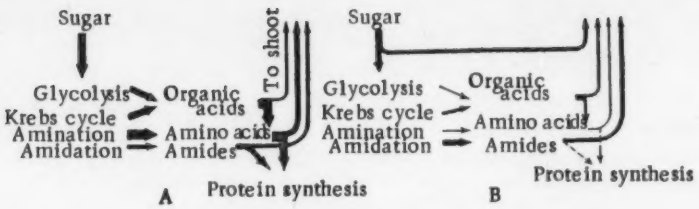

Fig. 5. Scheme for metabolic conversion in pumpkin roots.
A) Control. B) With soil drought.

An idea of the differences in external appearance of the plants is given by the photographs taken in 1957 (Fig. 1). In Fig. 1A the plants were photographed 28 days after treatment began and four days after the first samples were taken; in Fig. 1B after 48 days at the very end of a drought period. It is apparent that growth of the plants is very strongly retarded by drought which is also affirmed by the data of Table 1. All of the plants, regardless of the severity of the drought to which they were subjected, resumed growth after a liberal irrigation.

The dry weight of the root system during the initial period of drought still increased a little (though in some cases more slowly than in the control). Later its rate of dry weight increase fell progressively and in the 1958 experiment toward the end of the drought period even noticeably lost dry weight. During the initial part of the drought period the weight loss by the shoot was distinctly greater than in the control but toward the end of the drought period it invariably decreased in coincidence with a complete curtailment of growth and death of the lower and part of the middle leaves. The total amount of sap secreted during dessication diminished to $^2/_7 * ^1/_9$ (but in later stages of drought, when the soil moisture supply was about 20% of F.C., generally sap could be collected only when the plants were irrigated a little) and this is completely understandable.

More important here, however, is the fact that the total amount of sap, based on a unit dry weight of roots, (an index which to a recognized degree characterizes the productivity of the root system) decreased $^2/_5 - ^1/_7$; it returned to normal only after the drought was terminated.

As the soil moisture supply decreased much sugar accumulated in the roots since the total amount of sugar was several times greater than in the control plants; the specific content of sugars (especially fructose and glucose) toward the end of the drought period may exceed the control by 7-10 times. In addition, sugars (chiefly fructose and glucose) appeared in the sap while the sap of control plants usually did not contain them. The latter agrees with the data of other workers [15, 16]. But it was exceedingly characteristic that, when the drought was terminated and growth of the irrigated plants resumed, the total amount and specific content of sugars (also primarily glucose and fructose) in the root tissues rapidly decreased and sugars completely disappeared from the sap.

Inasmuch as the entire chain of metabolic transformation in the root system begins with sugars [10, 12, 14], the presumption naturally arises that there is a connection between sugar accumulation and a slowing down of carbohydrate conversions. In this event a lag in further conversions in the roots would also presumably cause a return of part of the unused sugars by way of the sap to the aerial organs. On the other hand, because the sugars are rapidly consumed the specific sugar content of leaves of normal plants in our experiments was many times larger than that of the roots and sugar was absent from the sap. It ought to be emphasized that during drought the specific sugar content of the leaves also increased (except, sometimes, for only the very top, juvenile leaves) and thus the circulation of carbohydrates from the leaves to the roots and from the roots back to the leaves clearly has little effect.

An investigation of the sugar conversion products — phosphorylated compounds, organic acids, and amino-acids demonstrated that the concept that sugar accumulation is connected with an impedance to sugar conversions is not an ill-founded idea.

It is primarily phosphorylation that is interrupted. Not only the total content in various tissues but also the specific content of phosphoorganic compounds in the acid-soluble fraction including phosphated sugar esters, ATP and a number of other active components of the respiratory metabolism decreases continuously in comparison to the control as drought becomes more severe (Fig. 2). If we compare the changes in phosphorylation products

with changes in the simple sugars, it is not difficult to see a great similarity with analogous changes detected earlier in the leaves [1]. In the leaves, likewise, an increase of the ratio between simple sugars and phosphorylated compounds indicates a probable weakening of the phosphorylation reactions, implying that the same disruption in the respiratory metabolism during drought occurs in not only the leaves but also in the roots. Especially meriting attention in this connection is the fact that the total ATP content decreases which in this case certainly indicates a weakening of the processes of accumulation and transport of respiratory energy. Apparently the retardation of phosphorylation does not depend simply upon the extent to which tissues are supplied with mineral phosphorus. It is true that the total amount of mineral phosphorus in many cases decreases (Fig. 2B). However, a decrease in the specific content of phosphorylated compounds has been noted even when mineral phosphorus content increases. For example, at the end of the drought in 1957 the ratio of mineral phosphorus to acid-soluble phosphorus compounds became larger than in the control plants (Table 2). Therefore, phosphorylation was depressed not only because of a mineral phosphorus deficiency but also for some other reason.

Disruption of the phosphorus metabolism proved to be so profound that it led to an appreciable decrease in the total amount of nucleic acid RNA (Fig. 3A) and DNA. The specific content of RNA and DNA also decreased (but to a lesser extent). The adverse effect of a water deficit upon the nucleic acid metabolism has been recently noted in the literature [26]. In view of the unconditional necessity of RNA for protein synthesis [27] it might be supposed that these facts relate directly to the suppression of protein synthesis which is detected among very early symptoms of plant water stress.

A suppression of protein synthesis is indicated, first, by an abrupt slowdown and then a complete cessation of growth of the root system and, secondly, by an appreciable decrease in the total protein content compared to the control plants (Fig. 3B). It is true that the latter condition could be explained by not only a decrease of protein synthesis but also by an increase in protein hydrolysis. However, in all probability an increase in protein hydrolysis does not occur. In any case, during drought the protein component of the total nitrogen is conserved for a long time or even increases (during drought 84-88%, control 75-80%). It decreases somewhat (to 77%) only toward the end of the drought period. However, the specific content of protein nitrogen decreases insignificantly (during drought it decreases from 28.8 mg in the initial period to 25 mg at the end of the experiment, in control plants it was about 30 mg/gm over-dry weight) and we cannot speak of proteolysis activity on the basis of these data.

The absence of an increase in hydrolysis during prolonged drought so typical of short-period wilting [19, 28, 29] has already been observed for example in the case of wheat leaves [30]. Whatever is the cause for the increase of the protein component during drought amid simple nitrogenous materials of the root, it results in a very strong decrease in the specific content of mineral nitrogen compounds.

However, if the total amount of proteins almost never increases, then the question arises: Why does the root dry weight rise during the initial part of the drought? This increase depends chiefly upon the accumulation of sugars. A substantial increase in the carbohydrate component of dry weight and some decrease in the specific content of proteins during drought can be demonstrated. That the increase of root dry weight during drought occurs because of a gain in carbohydrates and not proteins is evident, for example, from a comparison of the 1957 and 1958 data. In 1958, when the experimental plants were more severely suppressed and an earlier and more rapid curtailment of the assimilating surfaces occurred, carbohydrate accumulation in the roots did not appear to be as great as in 1957. Moreover, the increase in dry weight of the root system during the experimental period was comparatively smaller than in 1957 and during the late stages of drought the root system even lost dry weight.

In recalling the sequence of steps in further conversions of the sugars it is necessary to note that the interruption of phosphorylation, i.e., the process with which glycolysis begins, affects formation of the terminal products of glycolysis, pyruvic acid and acids of the Krebs cycle.

Malic, citric, succinic, fumaric and a few unidentified acids were detected in the roots and sap. The composition of the acids in the sap corresponded more or less to that in the roots. Malic acid quantitatively predominated in normal plants. The proportion of malic to the simple organic acids increased noticeably as the plant grew older. At the same time the roots of young plants were relatively richer in succinic and fumaric acids.

During drought the specific content of succinic and fumaric acids (not to mention the total amount) decreased so abruptly and significantly that we were not able to find it in either the roots or sap. The total amount of citric and malic acids in the roots decreased little at the beginning. At the same time the total amounts of

citric and malic acid in the sap (particularly malic acid) increased several times (this observation is of very great interest, however; we will return to an explanation of it later). Then, during a certain stage of the drought period (soil moisture about 20% of F.C.) citric and especially malic acid commenced to accumulate in the roots accompanied by a decrease in their concentration in the sap. The total amount of malic acid in the roots increased to six times that in the controls and citric acid increased two and one half times. Quite large amounts of an unidentified acid not detected in the control plants and disappearing when the drought ended also appeared in the sap. On the chromatogram its spot lay between succinic and fumaric acids (solvent butanol-fumaric acid-water). As drought increased, the total amount of malic and citric acid in the roots again decreased, this time very significantly. The total amount of unidentified acids in the roots during drought was also less than in the control plants.

Thus, during a sufficiently severe drought (about 15% of F.C.) the total amount of all identified organic acids decreased in the roots. It is typical that the water deficit primarily affected the specific content of fumaric and succinic acids, i.e., precisely those acids whose specific content is greatest in the roots of young plants. Apparently, the presence of these acids is linked to their greater metabolic role. On the other hand, during a certain drought stage there is present a large amount of malic acid which normally accumulates as plants grow older. Therefore, it can be hypothesized that during drought the root metabolism in certain respects shifts in the same direction as it does when the plants grow older. A comparison of the detrimental effect of drought with enforced aging of plants has been made by Alekseev [31].

From among the six keto acids which have been found in roots three have been identified: pyruvic, α-ketoglutaric and oxalacetic acids. Normally, α-ketoglutaric, pyruvic and one unknown acid (X), which lies between oxaloacetic and α-ketoglutaric acids on the chromatogram quantitatively predominates.

As drought became more severe the total amount of all acids gradually decreased with the exception of acid (X), the amount of which remained nearly unchanged. A decrease in the total amount of pyruvic acid is detected before the others. However, toward the end of the drought period a sudden difference occurred between the amount of α-ketoglutaric acid of the drought-treated plants and the controls. During earlier drought stages, on the other hand, the amount of α-ketoglutaric acid increased. Data on the total and specific amounts of pyruvic and α-ketoglutaric acids at the end of drought period are presented in Table 3.

The described disruption in organic acid metabolism must without question affect the formation of amino acids, a problem to which we will now turn our attention.

In the roots we have identified lysine, histidine, arginine, aspartic acid, serine, glycine, glutamic acid, threonine, alanine, proline, γ-amino butyric acid, tyrosine, tryptophan, valine, methionine, phenylalanine, isoleucine, leucine, and the amides glutamine and asparagine. Among the amino acids, alanine and glutamic acid quantitatively predominate. Glutamine is appreciably more abundant than asparagine. The amino acid composition of the sap corresponds to the amino acid composition of the roots.

As drought intensified, the total amount of amino acids gradually decreased. This is primarily with respect to alanine and glutamic acid, the total amount of which progressively decreased in the roots and sap beginning at a certain time during drought. Later, but just as markedly, the total amount of an overwhelming majority of the other amino acids decreased with the exception of methionine and threonine, the total amount of which in individual cases did not decrease.

Of course, the amino acid balance in the roots is comprised of many components (amino acid synthesis, their subsequent use for the synthesis of protein in the root, efflux from the root, flow into the shoot, deamination, proteolysis, etc.); however, in the case given here there is a basis for assuming that a decrease in the total amount of amino acids to a significant degree is explained by obstructions to their synthesis. The behavior of the amides and also of the organic acids serve as grounds for such a hypothesis.

During drought the amide component increased such that glutamine gradually began to assume first place among all of the compounds enumerated earlier. As soil moisture decreased to 20% of F.C. not only the proportion but also the total amount of amides increased in the roots and especially in the sap (this period coincides with the accumulation of malic and α-ketoglutaric acid). Toward the end of the drought period the total amide content of the roots somewhat decreased compared to the control, however their specific content remained the same or was a little greater than in the control plants whereas no analogous increase in the specific content of amino acids was observed. An increase in the proportion of amides in the sap was observed during the entire experiment right up until the time the wilted plants were irrigated, immediately after which, at the same time the plant growth resumed, the amide fraction suddenly became smaller (Fig. 4).

Until now it has been assumed as quite firmly established that first, roots are able to synthesize amides during the process of assimilating ammonium nitrogen [8, 13, 14], secondly, that amidation is accomplished more easily than amination [32], and, thirdly, that glutamine is not only the kind of compound that is linked to the ammonium ion entering the plant, but also serves as an exceedingly important source of amine groups for transamination with keto acids [33]. Considering all this, we might attribute the accumulation of amides in our experiments to their relatively more rapid formation due to their greater ease of amidation compared to amination, and thus the acquisition of amine groups in transamination reactions is blocked and the latter is inhibited (more quickly than the former). In both cases, however, we face the fact that the amination reaction is inhibited during drought.

A decrease in the total content of organic acids serving as acceptors for ammonia during amino acid synthesis can play a definite role. All comparisons of changes in the organic acids, amino acids, and amides indicate that an accumulation of amides and a decrease in the total amount of a whole series of amino acids begins before the decrease in specific content of organic acids, and, consequently, the crux of the matter here is not just one of a deficiency of suitable ammonia acceptors. For example, a decrease in the total content of alanine and glutamic acid and the accumulation of glutamine commences precisely at that time when the total amount (not to mention the specific content of α-ketoglutaric acid content) is equal to the specific content of pyruvic acid just as the specific content of pyruvic acid was greater than in the control plants. Despite a decrease in its total content, even during intense drought the specific content of pyruvic acid remained high (Table 3), and consequently, it is impossible to explain the abrupt reduction in total alanine content of the roots and sap in any other way than as a block in the amination of pyruvic acid or even partly by deamination of earlier synthesized alanine. It is apparently quite true that under droughty soil conditions there is a severely retarded absorption of ammonium ions, an insufficiency of which could in turn obstruct amination. At the same time, however, amidation is not in the least reduced and ammonium is sufficient for an accelerated formation of amide groups. Finally, a decrease in the total amount of amino acids was observed under conditions where there was an sharp decrease in their consumption for protein synthesis. All of this indicates an obstruction in the formation of new amino acids linked not only with simply a deficiency of suitable ammonium acceptors and ammonia itself. It is interesting that a similar picture of blocks in the amination of keto acids and an increase in the proportion of amides occurs in phosphorus deficient roots [14].

The data presented on carbohydrates, phosphorylated compounds, organic acids, and amino acids conforms to the hypothesis that soil drought restricts the conversion of sugars flowing from the leaves by blocking reactions in glycolysis, the Krebs cycle and amination, which also explains the observed marked sugar accumulation in the roots and the return of part of the unused sugars by way of the sap to the shoot. The shoot, being saturated with sugars, scarcely needed the sugars transferred from the roots. Thus, a pathological circulation of sugars was engendered. Metabolic changes in the roots are illustrated by the diagram (Fig. 5).

The sequence of changes which occur may not be useless for clarification of the direct causes of the disruption of the metabolism. During the earliest stages of drought the total amount of many amino acids and organic acids increased in the roots and sap (particularly the latter). However, and this is very important, both the total and the specific content of phosphorylated products had started to decrease during this period. Protein synthesis was blocked early and sugars appeared in the sap. Therefore, the normal synthesis activity of the roots had been interrupted by that time, the phosphorylation and protein synthesis processes being obstructed first. In the light of all this (if we consider that proteolysis does not increase in either the roots or the leaves) the initial accumulation of organic acids and amino acids is basically explained by a decrease in their further utilization by the roots because growth is inhibited and not because amino acid synthesis is increased. Evidently, precisely because of a decrease in amino acid utilization, the amides and organic acids during the initial period of drought not only accumulate in the root tissues but, as has been mentioned during the course of this account, are precipitated from the sap in relatively still large amounts.

Changes in composition of the sap merit detailed examination since we here encounter a question of great fundamental importance: How much can be judged from the composition of the sap concerning the synthesis activity of the root system? However, although during the earliest parts of the drought period the influx of many amino acids and organic acids with the sap noticeably increases, we have no basis for connecting this event with the simultaneous intensification of specific biosynthesis. On the other hand, there are weighty arguments for concluding that a slowdown occurred in regeneration of the indicated substances in the roots. It is also characteristic that the tempo of secretory desorption into the xylem vessels to a great extent determines the tempo of accumulation

of the organic acids and amino acids in the root tissues. Because of the accelerated secretion of nitrogen-containing organic materials with the sap and because of a simultaneous, significant decrease in the specific content of nitrates and ammonia, the ratio between organic and mineral nitrogen compounds in the sap during this period shifts toward the side of the organic compounds. The same thing was found by Kulaeva [14] in phosphorus-deficient pumpkin. For a correct assessment of these facts it is necessary to keep the following in mind. It has been already quite definitely demonstrated that the supply of the products of root nutrition to the aerial organs can actually be judged by the total amount of substances secreted with the sap [4-7, 14, 34]. The possible participation of phloem elements in the ascending stream does not alter the proposition, inasmuch as when the phloem is breached the stream of substances formerly controlled by the phloem is diverted to the xylem and, therefore, the sap collected is not different. In addition to this, the total amount of substances secreted with the sap is determined not by the requirements of the aerial organs for these substances but by the metabolism of the roots themselves [17].

Although in a number of cases the specific content of mineral nutritional elements in the sap correlates quite well with the rate at which they are absorbed from the external medium, to judge the absorption of substances on the basis of sap composition is completely impossible. As Sabinin has emphasized [5], the ratio $\frac{\text{secreted in the sap}}{\text{absorbed from the external medium}}$ is determined not only by the intensity of the absorption processes but also by the extent to which the absorbed substances are used in the root tissues. The latter determines their release from a number of labially linked components of protoplasmic compounds which are desorbed into the conducting traces of the central cylinder. Precisely because of this situation in the energy metabolism of growing root systems (i.e., ones having many requirements) the ratio above may be appreciably less than unity. A weakening of the "binding" power of the root cell protoplasm (for example, when the plant grows older) [4, 6, 36] may lead to an increase in the proportion of substances desorbed into the vessels of the control cylinder.

Now it is not difficult to devise an analog for secretion with the sap of organic substances synthesized in the root cells. Despite the fact that there is still very little known about the factors which determine the proportion of substances secreted with the sap, by the very nature of this secretion it is evident to everyone that the $\frac{\text{secreted in the sap}}{\text{synthesized in the root}}$ is determined not only by the rate of biosynthesis but also by the requirements of the root itself for synthesized products, primarily for the proteins synthesized. A slowing-down of growth processes (and that means the bases for protein synthesis) may weaken the "binding" ability of root tissues with respect to substances ordinarily used as structural materials and may lead to an increased desorption of these substances into the xylem vessels, i.e., just as we observed during the very early stages of drought and partly also during the later stages (with respect to amides). Therefore, it is quite apparent that to judge the synthesis capability of root systems on the basis of sap composition alone, as has been widely done in the past (for example, on the basis of the ratio of the organic to mineral form of nitrogen), is quite risky especially in studies of the effect of various unfavorable factors closely associated with changes in the ratio of growth. In order to come to a conclusion regarding the synthesis ability of root systems, the analysis of sap must be matched with a thorough analysis of the root system itself keeping in mind the condition of the plant as a whole.

In our experiments sap composition appeared to be a very sensitive criterion for the "binding" ability of the root cell protoplasm with respect to sugars, amino acids, amides, and organic acids, since it graphically reflects any sharp decrease in the use of these substances within the roots under conditions when growth and protein synthesis is suppressed. However, we were not able to detect phosphoorganic compounds in the sap of plants subjected to drought. This is also one of the indications that suppression of phosphorylation begins during very early phases of drought, markedly earlier than the disruption of the synthesis of amino and organic acids. We have discussed this and other data presented above.

Thus, the suppression of phosphorylation indicating a disruption of the energy metabolism is, in addition to the obstruction of protein synthesis, a very early symptom of plant moisture stress. Probably, therefore, for just this reason, there are many features in common between changes in root metabolism during drought and phosphorus deficiency since in both cases we have to deal primarily with a disruption of the phosphorus metabolism. Such an unusual aspect of phosphorylation is fully understandable if we consider its role to be the first step in the energy metabolism of organisms, the very basis of vital activity [1, 37].

We also observed similar disturbances in the root metabolism of wheat and cucumbers during drought in greenhouse experiments in 1955-1957.

SUMMARY

1. Prolonged and severe soil drought retarded and then put a stop to plant growth leading to a serious disturbance in the metabolism of the root system and a decrease in its productivity. Glycolysis, Krebs cycle, and amination reactions were slowed down. The total content of acid-soluble phosphoorganic compounds (among them ATP), nucleic acids, many organic acids and amino acids was reduced. Typically, a decrease was observed in the specific content of phosphorylated compounds accompanied by an increase in the specific content of mineral phosphorus in the root tissues. Protein synthesis was observed in either the leaves or roots. As a consequence of the retardation of translocation into the roots, a large total amount of sugars accumulated and a part of the unused sugars was returned in the sap to the shoot. The specific content of these same carbohydrates in the shoot was thus increased and transfer of the sugars from the roots was therefore scarcely necessary.

Thus, a pathologic carbohydrate circulation developed in the plant. The total amount of amino acids in the sap gradually decreased. Because of the obstruction of amino acid synthesis the amide fraction in the roots and sap grew larger.

2. Just as has been shown earlier for leaves, an increase in the ratio between simple sugars and phosphorylated compounds in the roots indicates a weakening of phosphorylation reactions, implying a disturbance in the energy metabolism itself. The depression of phosphorylation besides being an obstruction to growth and protein synthesis is the very earliest symptom of plant water stress.

3. The presence of the increasing total amounts of organic compounds in the sap during the initial stages of a drought period reflects not a corresponding increase of synthesis in the roots but rather a decrease in the ability of the protoplasm in the root cells to hang onto these substances because of a weakening of synthesis activities. The ratio secreted in sap synthesized in root is, obviously, determined not only by the intensity of biosynthesis but also by the rate at which the synthesized products are used by the root system itself. Therefore, in order to come to an conclusion concerning the nature of the synthesis activity of the root system, especially in studies of the effect of unfavorable factors accompanied by marked changes in growth rate, a sap analysis msut be compared with root analysis taking into account the condition of the plant as a whole.

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LITERATURE CITED

[1] V. N. Zholekevich, Doklady Akad. Nauk SSSR 121, 6, 1093 (1958). •

[2] N. S. Petinov, Biological Bases for Irrigated Agriculture [in Russian] (Izd. AN SSSR, 1957).

[3] N. S. Petinov and K. M. Korshunova, Fiziol. Rast. 4, 365 (1957). •

[4] D. A. Sabinin, Mineral Nutrition of Plants [in Russian] (Izd. AN SSSR, 1940).

[5] D. A. Sabinin, Significance of the Root System in Plant Vital Activities, Timiryazev Lectures IX [in Russian] (Izd. AN SSSR, 1949).

[6] D. A. Sabinin, The Physiological Basis for Plant Nutrition [in Russian] (Izd. AN SSSR, 1955).

[7] N. G. Potapov, Investigations of the Intake of Nutrient Substances [in Russian] Diss. VIUA (1936); Vestn. Agrotekhniki VASKhNIL, No. 2, 71 (1940); Tezisy Dokl. Delegatskogo S"ezda VBO, 100, Leningrad (1957).

[8] R. MacVicar and R. J. Burris, Biol. Chem. 176, 511 (1948).

[9] S. Aronoff and L. Vernon, Arch. Biochem. and Biophys. 36, 383 (1952).

• See English translation.

[10] A. L. Kursanov, Vestn. AN SSSR, No. 12, 26 (1953); Vopr. Botaniki 1, 131 (1954); Fiziol. Rast. 2, 271 (1955); Izv. AN SSSR Seriya Biol. No. 6, 689 (1957).

[11] F. M. Turchin, M. A. Guminskaya and E. G. Plyshevskaya, Fiziol. Rast. 2, 3 (1955).

[12] N. A. Pristupa and A. L. Kursanov, Fiziol. Rast. 4, 417 (1957). *

[13] E. W. Yemm, Recent Development in Cell Physiology (Bristol, 1954) p. 51.

[14] O. N. Kulaeva, Metabolism of Substances in Pumpkin Roots in Relation to Assimilation of Ammonia Nitrogen, Diss. [in Russian]. Inst-a Fiziol. Rast. AN SSSR (1959).

[15] L. S. Litvinov, Izv. Biol. n.-i Inst-a i Biol. Stantsii pri Permskom Gos. Un-te 5, 311 (1927).

[16] J. Dagys, Protoplasm 24, 14 (1935).

[17] O. F. Tueva and S. A. Samoilova, Trudy Inst-a Fiziol. Rast. AN SSSR 6, 118 (1948).

[18] A. L. Kursanov, O. F. Tueva and A. G. Vereshchagin, Fiziol. Rast. 1, 12 (1954).

[19] O. M. Trubetskova and O. S. Semenova, Trudy Komissi po Irrigatsii AN SSSR, No. 8, 95 (1936).

[20] A. M. Boyarkin, Fiziol. Rast. 2, 298 (1955).

[21] N. P. Meshkova and N. V. Aleksakhina, Achievements of Biological Chemistry, [in Russian] (Izd. AN SSSR, 1954).

[22] A. M. Sobolev and E. I. Vyskrebentseva, Fiziol. Rast. 6, 244 (1959). *

[23] A. N. Belozerskii and N. I. Proskuryakov, Practical Handbook of Plant Biochemistry [in Russian] (Izd. Soc. Nauk, 1951).

[24] A. N. Boyarkin and M. I. Dimitrieva, Fiziol. Rast. 5, 386 (1958). *

[25] M. G. Zaitseva, Study of Cell Growth during the Elongation Phase as a Basis for the Formation of Succulent Structure, Diss. [in Russian] (MGU, 1957); Bot. Zhurn, 36, 288 (1951).

[26] C. T. Gates and J. Bonner, Plant Physiol. 34, 49 (1959).

[27] N. Borsuk, Contemporary Problems in Biochemistry, [Russian translation] (II, Moscow, 1957).

[28] K. Mothes, Ber. deutsch. bot. Ges. 46, 59 (1928).

[29] N. M. Sisakyan, Biochemical Characteristics of Drought-Resistant Plants [in Russian] (Izd. AN SSSR, 1940).

[30] V. N. Zholkevich, Fiziol. Rast. 2, 123 (1956); The Biological Bases of Irrigated Agriculture, [in Russian] (Izd. AN SSSR, 1957).

[31] A. M. Alekseev, Plant Water Regime and the Effect of Drought Upon It [in Russian] (Tatgosizdat, Kazan,' 1948).

[32] V. L. Kretovich, Izv. AN SSSR, Seriya Biol. No. 2, 129 (1958).

[33] V. L. Kretovich and V. I. Yakovleva, Fiziol. Rast. 6, 165 (1959). *

[34] O. N. Trubetskova and N. G. Zhirnova, Fiziol. Rast. 6, 129 (1959). *

[35] E. I. Ratner, T. A. Akimochkina and S. F. Ukhina, Fiziol. Rast. 6, 3 (1959). *

[36] O. F. Tueva, Izv. Biol. n.-i Inst-a i. Biol. Stantsii pri Permskom Gos. Un-te, 6, 261 (1929).

[37] "Phosphorylation and function," Tezisy Dokl. Inst-a Éksperium. Meditsiny AMN SSSR, Leningrad (1958).

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* See English Translation.

THE CONNECTION BETWEEN PHOTOPERIODISM AND RESPIRATION IN PLANTS

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The photoperiodic reaction of flowering is accomplished with the action and interaction of all organs of the plants, but the organ immediately absorbing the photoperiodic reaction is the leaf. Respiration takes a direct part in the processes of the photoperiodic reaction taking place in the greenleaves of plants [1, 2].

The relation of respiration to the dark processes taking place during the long-night period of the short-day cycle has been studied best of all. These dark processes accelerate flowering of short-day species and prevent flowering of long-day species. Influences during the long night, such as the setting of plants in anaerobic conditions [3, 4] and the supplying of leaves with a supplementary quantity of sugars as a respiratory substrate [5], essentially change the period of the dark processes of photoperiodism. Apparently these same influences also lead to substantial changes in the process of respiration. The first test in the process of solving the question of which links of the respiratory process take part in the dark reactions of photoperiodism was made in an experiment with the short-day varieties of morning glory (Pharbitis) [6]. It appeared that when the cotyledons of young plants were immersed for one day in solutions of inhibitors of respiration at the time of the dark period, the flowering of this short-day species is inhibited by cyanide, azide, arsenite, and fluorine salts, but is not inhibited by malonate even in very high concentrations. This experiment indicates the participation of the processes of glycolysis, oxidizing phosphorylation and of enzymes containing heavy metals in the dark reactions of photoperiodism for Pharbitis.

Up to now there have been no direct indicators of the immediate effect of the process of respiration in the light reactions, which take place during the light hours on the long day, and also in the light reactions with the interruption of the long night by light flashes. However, in connection with the effect of the length of the day, significant changes were also established in the intensity of respiration in the light hours of the day. Thus, in the leaves of soy and xanthium (short-day species), the intensity of respiration measured against an average day increased, and for barley (long-day species) decreased to the extent of the passing of the photoperiodic reaction [7]. It was also shown [8] that the intensity of respiration of short-day and long-day plants characteristically is changed when the long night is interrupted with short flashes of red and infrared light, increasing with the action favorable for development and decreasing with unfavorable action. Thus, judging by the results of these works, respiration fulfills a very different role in the photoperiodic reaction, sometimes taking part in the dark processes of photoperiodism and characteristically changing in the light hours of the day and also when interrupted during the night with short flashes of light.

However, the concrete functions of respiration in the photoperiodic reaction remain unknown. In the same way, very little is known about which links and enzyme systems of the complex and multistaged process of respiration have an immediate relation to photoperiodism. This makes further study of the relation of respiration to the photoperiodism of plants necessary.

1. Effect of the Length of Day on the Diurnal Dynamics of the Intensity of Respiration

Photoperiodism consists of a number of processes taking place during the day in a certain order. This means the processes of metabolism, which are connected with the mechanism of the photoperiodic reaction, must also have sufficiently clearly expressed diurnal dynamics, differing with different day lengths.

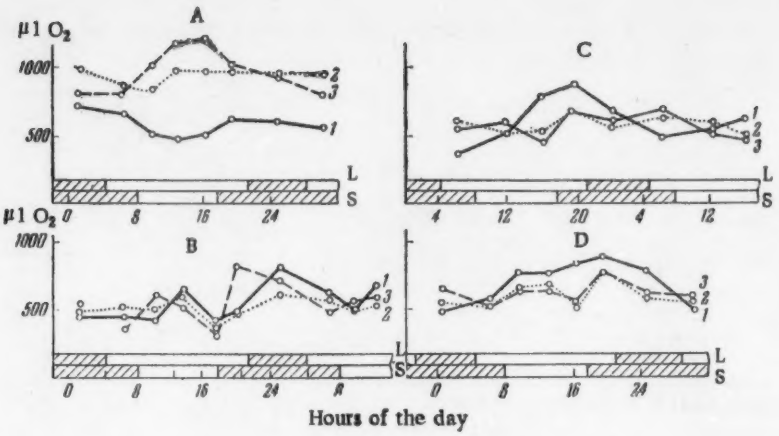

Fig. 1. Diurnal dynamics of the intensity of respiration of rudbeckia (A), oats variety pobeda (B), perilla (C), and Japanese millet (D) on long and short-day (in µl O_2 per hour per 100 cm^2 of leaves). Rudbeckia: 1) 17 long days, shoot formation; 2) 3 long days, rosette; 3) short day, rosette; experiment on July 18, 1957. Pobeda Oats: 1) 30 long days prior to spike formation; 2) 10 long days, head formation; 3) short day, head formation, June 29, 1957. Perilla: 1) long day, vegetative growth; 2) 10 short days, prior to bud formation; 3) 24 short days, bud formation; experiment July 9, 1957. Japanese millet: 1) long day, stem formation; 2) 3 short days, stem formation; 3) 18 short days, flower formation; experiment July 30, 1957. Crosshatched) dark; not crosshatched) light; L) long day; S) short.

In 1957 when studying the character of the relationship between the process of respiration and photoperiodism, we began with an explanation of the diurnal dynamics of the changes of the normal intensity of respiration on the different lengths of day for two long-day species, Rudbeckia (Rudbeckia bicolor) and Pobeda oats (Avena sativa), and for two short-day species, red perilla (Perilla nankinesis) and Japanese millet (Panicum miliaceum).

We compared the respiration of plants on the long day, unfavorable for development, and on the favorable day at the time of the photoperiodic reaction taking place in the leaves. The natural Moscow winter day to which was added light of an incandescent lamp, served as the long day. The short 10-hour day was achieved by means of putting plants in light-proof chambers from 6 P.M. to 8 A.M. of the following day. All plants were grown on an unfavorable-length day during a period of one to three months (depending on species) and then some of the plants were placed on a favorable-length day for three to seventeen days prior to the experiment.

The intensity of respiration was determined by the manometric method at 25° for an average test section from the fully formed leaves which had completed growth.

The results of four of the eighteen experiments that were carried out are shown in Fig. 1. The remaining experiments gave similar results.

As we can see from the indicated graphs, the photoperiodic action showed a noticeable effect on the dynamics of the changes in the intensity of respiration over the period of 24 hours. The dynamics of the intensity of respiration for all plants had a different path on the short day than on the long day. The minimum and maximum points of the intensity of respiration over time do not coincide for the different lengths of day, and therefore the relationship of the intensity of respiration of short and long day variants also is sharply changed over the 24-hour period (for example, see Fig. 1, B and C). The relatively rapid (not more than three days; see Fig. 1, A and D) change in the rhythm of the intensity of respiration under the effect of a change in the length of day can be related mainly to the degree of the action of a different duration of light and dark on the short and long day, because the remaining conditions, temperature, moisture, and intensity of lighting, differed insignificantly for the two photoperiods.

TABLE 1

Change in the Activity of the Oxidase in Relation to the Length of Day (average of six to eight measurements per day)

Date	Plant	Photoperiod	Absorption of O_2 µl per 15 minutes per 100 cm² of leaves			Absorption of O_2 as % of normal •	
			Respiration				
			normal	remaining	inhibited	remaining	inhibited
Short day species							
Sept. 6 - 7	Kharbin soy	Long day	195	59	136	29	71
		Three short days	186	88	98	47	53
July 5 - 6	Japanese millet	Long day	196	112	84	57	43
		Five short days	170	132	38	72	28
Aug. 10 - 11	Mamont tobacco	Long day	158	66	92	42	58
		Five short days	175	92	83	52	48
Long day species							
July 19 - 20	Rudbeckia	Short day	168	78	90	46	54
		Three long days	167	69	98	41	59
July 23 - 24	Rudbeckia	Short day	147	92	55	63	47
		Seven long days	202	49	153	24	76
July 11 - 12	Pobeda oats	Short day	115	28	87	24	76
		Three long days	149	10	139	6	94
July 16 - 17	Pobeda oats	Short day	126	38	88	30	70
		Seven long days	144	12	132	8	92
July 23 - 24	Silvestris tobacco	Short day	149	23	126	15	85
		Seven long days	153	4	149	2	98

• Normal respiration taken as 100%.

In a number of experiments, the clear effect of the change in light and dark on the intensity,of respiration can be observed directly. Along with the maximum respiration in the middle of the light period of the day, an increase is also often observed in the intensity of respiration in the dark; sometimes the rhythm of respiration has the character of a two-phase curve with two maximums, dark and light. Such a two-phase rhythm of respiration was noted, for example, for millet on a short day (Fig. 1 D) and for oats on a short and long day (Fig. 1 B). In other cases, for example with rudbeckia (Fig. 1 A), although there was no increase in the intensity of respiration in the dark, there was also no decrease in respiration at night on the long day in spite of the fact that during the night the temperature of the air always decreased noticeably (6-10°).

Thus, the change in the duration of lighting over the period of the day leads to a change in the diurnal dynamics of the intensity of respiration for all plants.

2. The Effect of the Length of Day on the Diurnal Dynamics of the Activity of the Oxidase Systems

Because the transfer of plants from an unfavorable length day to a favorable length immediately leads to a change in the diurnal path of the normal intensity of respiration as measured by the absorption of oxygen, it is interesting to follow in more detail the character of this change in respiration. The enzymes responsible for the absorption of oxygen in the process of respiration are terminal oxidases. Therefore, in the experiments carried out in the summer of 1958 at the time of the photoperiodic induction for a number of short day and long day plants, the diurnal dynamics were traced for the activity of the two large groups of oxidase systems: the group of enzymes containing heavy metals and inhibited by poisons of the type of azide and cyanide, and the group of enzymes of the residual respiration.

The activity of the residual respiration was measured by the absorption of oxygen by slices from leaves infiltered with a water solution of sodium azide of a specially selected concentration (in the range of 0.005 M

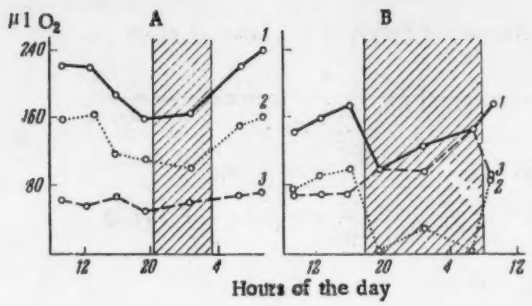

Fig. 2. Diurnal dynamics of the activity of oxidases for rudbeckia (in µl O_2 per 15 minutes per 100 cm^2 of leaves). A) Seventh day of induction by long day; B) short day; 1) normal respiration; 2) inhibited part of respiration; 3) residual respiration. Crosshatched part is dark, noncrosshatched is light.

up to 0.015 M for the various species and age conditions of the plants). The respiration of sections of leaves infiltered with water served as a control. The activity of the absorption of oxygen in proportion to the oxidases containing heavy metals was estimated by the differences in the intensity of respiration of the control leaves and leaves infiltered with the azide solution. In the primary experiments, we found that for our objects the suppression of respiration with sodium azide and cyanide has a similar character. We conditionally related the dynamics of the inhibited and residual respiration to the degree of change in the activity of the corresponding oxidase systems, although the method of inhibition with azide of whole pieces of leaves, as also every other method of measuring enzyme activity of living plant cells, has many limitations [9], and is rather an indirect method of measuring the activity of the oxidase systems.

The absorption of oxygen was calculated by the manometric method in a Warburg instrument at 30°. All samples taken during the dark period of the day were treated in a dark room and all samples taken in the light hours were treated in the light.

Long day species Pobeda oats, rudbeckia and silvestris tobacco (Nicotiana silvestris) and short day species, Japanese millet, Kharbin soy (Soja hispida) and Mamont tobacco (Nicotiana tabacum) were used as objects for the experiments. We compared the diurnal path of the oxidase activity in the leaves of plants in the unfavorable-long day and in leaves of plants on the third and fifth - seventh day induction of the long day, favorable for the intensive flow of the photoperiodic reaction.

We can see from the graphs (Figs. 2-6), that the photoperiodic effect primarily shows a specific effect on the normal level of the activity of the oxidase systems. For all six species of plants, both the long day and short day, on the long day a higher activity of the oxidases inhibited by azide is observed, while on the short day the normal level of the activity of the oxidases of the residual respiration is increased (Table 1).

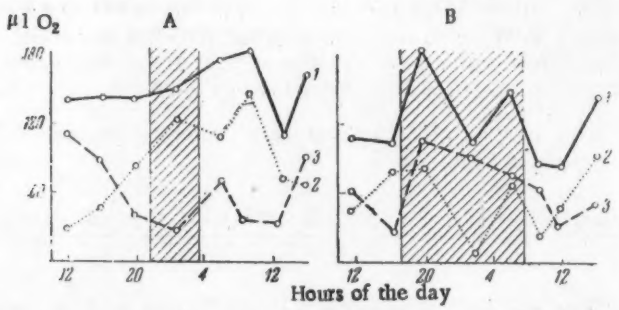

Fig. 3. Diurnal dynamics of the activity of oxidases for Mamont tobacco (in µl O_2 per 15 minutes per 100 cm^2 of leaves). A)Long day; B) fifth day of induction by short day; remaining figures as in Fig. 2.

The share of the part of the oxidases of the residual and inhibited respiration in the normal absorption of oxygen at the various hours of the day also differed. During the period of the day a change in the activity of the oxidase systems takes place in the process of respiration of the leaves of grown plants, often reaching significant dimensions. In a number of cases the size of this change of the oxidase systems suggests changes in the activity of the oxidizing enzymes which take place in the ontogeny of plants [10, 11], but it proceeds most in-

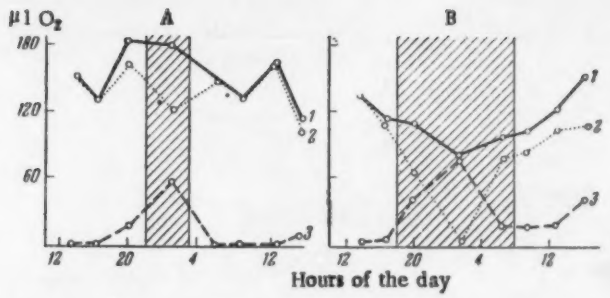

Fig. 4. Diurnal dynamics of the activity of oxidases for Pobeda oat (in µl O_2 per 15 minutes per 100 cm^2 of leaves). A) Third day of induction by long day; B) short day; remaining figures as in Fig. 2.

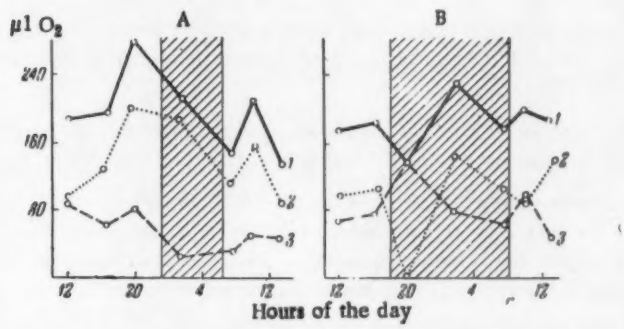

Fig. 5. Diurnal dynamics of the activity of oxidases for Kharbin soy (in µl O_2 per 15 minutes per 100 cm^2 of leaves). A) Long day; B) third day of induction by short day; remaining figures as in Fig. 2.

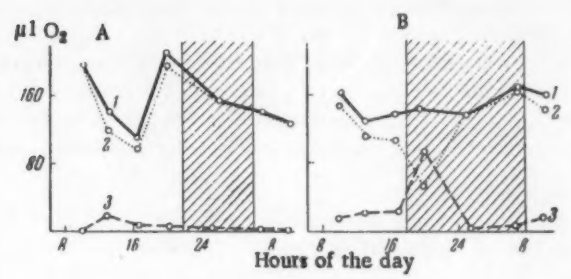

Fig. 6. Diurnal dynamics of the activity of oxidases for Silvestris tobacco (in µl O_2 per 15 minutes per 100 cm^2 of leaves). A) Seventh day of induction by long day; B) short day; remaining figures as in Fig. 2.

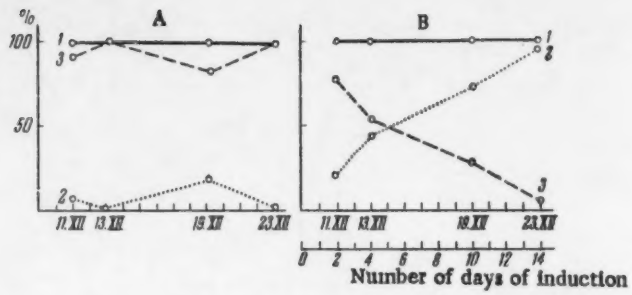

Fig. 7. The change in the activity of oxidase for rudbeckia at the time of induction by long day (as % of normal intensity of respiration). A) Short day (control); B) long day; 1) normal respiration; 2) inhibited part of respiration; 3) residual respiration.

tensively only after the passage of several hours. For example, for oats on the short day (Fig. 4) the action of the enzymes of the residual and inhibited respiration in the normal absorption of oxygen was changed over the passage of the day from 0 to 100%.

The shift in the activity of the oxidases of the residual and inhibited respiration have a certain diurnal rhythm, clearer on the artificial short day with the sharpshift of light and dark and less clear on the long day.

On the short day, both for long day (Figs. 2, 4, 6) and for short day (Figs. 3, 5) species of plants, with the onset of darkness there is a noticeable decrease in the activity of the oxidases inhibited by azide and simultaneously the intensity of the residual respiration is increased sharply. The large share of the part of the residual respiration in the normal respiration of the leaves is sometimes maintained up to the start of lighting (Figs. 2 and 3), and sometimes falls again during the second half of the long night (Figs. 5 and 6).

On the long day characterized by a long light period, no noticeable increase was observed in the residual respiration even during the time of the short night and the entire path of the activity of oxidase systems appears to be more level and even, in particular for long day species (Figs. 2 and 6) phylogenetically adapted to the long day photoperiod. The change in the diurnal dynamics of the activity of the oxidases under the effect of a changed length of day takes place very easily (Figs. 4 and 5). Only two days (the experiments were carried out on the third day) after plants of oat and soy were transferred from a length of day that excludes the passage of the photoperiodic reaction to a favorable length of day, the enzymes for oat suppressed by the azide began to be noticeably activated and for soy in the first half of the long night the intensity of the residual respiration increased. Such rapidity of changes in the diurnal dynamics of the oxidase activity on a change in the length of day suggests that the diurnal path of the oxidase systems is developed under the direct effect of the length of the day as such, and is not a result of a changing rate of the growth processes or of the processes of the differentiation of organs of reproductive development.

Thus the photoperiodic regime shows a strong effect both on the normal level of the activity of the studied groups of the oxidase systems and also on the diurnal dynamics and dimensions of the changes in the activity of each of these groups of enzymes.

3. The Change in the Activity of the Oxidase Systems At the Time of Photoperiodic Induction

In the winter of 1958-1959 the relationship of the activity of the oxidase systems was determined periodically for plants of a long day species, rudbeckia, and a short day species, Kharbin soy, at the time of a 14-day induction by favorable photoperiods. Measurements were carried out one time per day at the middle of the light period of the day. We considered the appearance of shoots on redbeckia on the 13 and 14th days of induction and the appearance on the 10th to 14th days of induction of incipient buds noticeable to the unaided eye on soy to be signs of the fact that the photoperiod reaction in the leaves was completed and the plants had moved toward generative development.

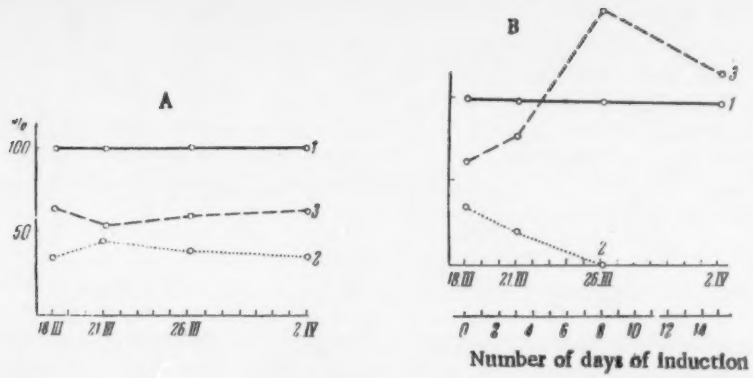

Fig. 8. The change in the activity of the oxidases for soy at the time of induction by short day (as % of normal intensity of respiration). A) Long day (control); B) short day; remaining figures as in Fig. 2.

The data obtained are presented in Figs. 7 and 8.

Based on the degree of increase in the number of days of action by the long day, the share of the part of the enzymes of the residual respiration in the normal absorption of oxygen for rudbeckia gradually dropped from the 80-100% characteristic for the respiration of leaves on the short day, to 0-10% of the fourteenth day of action by the long day. In accordance with this, the share of the part of the metal-containing oxidases gradually increased from 0-20% to 90-100% at the time of the completion of the photoperiodic reaction.

For a short day species, Kharbin soy, a gradual decrease in the part of the oxidases inhibited by azide takes place at the time of the photoperiod induction by the short day, along with an increase in the share of the residual respiration. Toward the time of the completion of the photoperiod reaction, infiltration by a solution of sodium azide leads not to an depression, but to an increase in the absorption of oxygen; that is, respiration practically proceeds according to the type of residual respiration.

Thus, at the time of the photoperiod induction both for short day and long day plants, a summation of the action of the separate favorable photoperiods on the activity of the corresponding oxidase systems is observed.

DISCUSSION

Light and dark are important factors showing an effect on the possibility of the existence of a photoperiod reaction for short day and long day species.

Suggestions that light and dark show an essential effect also on the mechanism of the chlorophyll-carrying tissues of the plant are presented in a number of works [12, 13, 14]. The change in the diurnal dynamics of the normal intensity of respiration of long day and short day species in relation to the duration of the light and dark on the passage of the day, and also the presence in the diurnal path of respiration of dark maximums not connected with fluctuations in air temperature, can be viewed as indirect support of these propositions. The effect of light and dark on the process of respiration is still more clearly shown in the shift of the activity of oxidase systems during the day and night. With a sharp shift from full daylight to darkness when the plants on the short day are placed in the light-proof box, the residual respiration was strongly activated in all experiments, while the long day activated the oxidases containing heavy metals.

The division of the oxidase systems into enzymes of the inhibited and residual respiration is a very normal division, and for various species of plants various enzyme systems apparently enter into these groups. Nevertheless, these two large groups of oxidizing enzymes on the whole are connected with the activation of different physiological processes and with the different directing of metabolism in the plant organism [10, 15-19]. Therefore, the intense shift of the activity of these large groups of oxidase systems in light and in the dark can be viewed as a reflection of the deep change in the process of respiration itself, and in a number of physiological processes connected with it that take place in the leaves of plants during the day and night periods for the short day and long day photoperiod.

Right now it is still difficult to say which of the concrete enzyme systems, including the inhibited oxidase and the oxidase of the residual respiration, are activated by light and dark in the leaves of mature plants. It has been shown [20, 21, 22 and others] that under the effect of light, especially short wave, the cytochrome oxidizing system is noticeably activated. Auxinoxidase is also strongly activated by light. Other authors, on the other hand, observed activation of polyphenoloxidase, the cytochrome system, and peroxidase in the dark [23] and on the short day [24] as compared with light and long day. Data on the action of light on the enzymes of the residual respiration are also contradictory. Therefore, the question of concrete oxidase systems activated during the day and night for short day and long day cycles remains as the object of further investigation.

The effect of short and long days on the relation of the activity of the oxidase systems for all plants, both for short day and for long day, is manifested in a similar manner. For all plants, long darkness on the short day on the whole activates the residual respiration and a long period of light on the long day activates the activity of the oxidases inhibited by sodium azide. Along with this, the sum of the effect of the separate favorable photoperiods on the relation of the activity of the oxidase systems takes place at the time of photoperiod induction. As a result, for long day species of plants, induction by the long day is accompanied by an intense increase in the activity of the inhibited oxidases, just as for short day species induction by short day is accompanied by an increase in the volume of the processes connected with strengthening the residual respiration. Consequently, at the time of the photoperiod reaction of flowering, different groups of the oxidase systems are activated in the leaves of short day and long day species of plants.

This result agrees with the proposition that even flowering of short day and long day species of plants is connected with the synthesis of various substances of a hormone-complex [25] and, therefore, also with the activation of the dissimilar processes of metabolism. Differences in the external conditions and, in particular, in the nutrition conditions aiding or inhibiting flowering of plants of different photoperiod groups suggest the fact that in the process of the photoperiod reaction several more processes of metabolism take part for short day plants than for long day species. Thus, the shift of long day species from vegetative to generative development aids in the moderation of the nitrogen starvation and in the additional supply with sugars, while for short day species these conditions of nutrition lead to the checking of the shift into the reproductive phase of development [5, 26, 27].

In connection with this, the proposition that different phases of the process of respiration take part in the photoperiod reaction leading to flowering for long day and short day plants is suggested as probable. Apparently, for short day species, mainly those links of the respiratory process that are activated by light and are connected with the strengthening of the activity of groups of oxidizing enzymes containing heavy metals, take part in the photoperiod reaction of flowering, while in the photoperiod reaction of short day species, very likely, mainly those links of respiration of the green leaves that are activated in the dark, and which are connected with the strenghtening of the residual respiration, take part.

SUMMARY

1. A change in the photoperiod regime leads to a rapid change in the diurnal dynamics of the normal intensity of respiration and in the diurnal dynamics of the activity of the various groups of oxidase systems.

2. The length of the day acts on the relation between the activity of the metal-containing oxidases and the oxidases of the residual respiration for short day and long day species of plants in a similar manner. Prolonged darkness for short day plants activated the residual respiration, while a long period of light in the long day activates the activity of the oxidases inhibited by sodium azide.

3. At the time of the photoperiod reaction of flowering, different oxidase systems are activated in the leaves of short day and long day species of plants. For long day species, induction by the long day is accompanied by an increase in the activity of the oxidases containing heavy metals, but for short day species induction by the short day is accompanied by a strengthening of the intensity of the residual respiration.

LITERATURE CITED

[1] M. Kh. Chailakhyan, Botan. Zhurnal 41, 4, 487 (1956).

[2] M. Kh. Chailakhyan, Zhurnal Obshch. Biol. 17, 2, 121 (1956).

[3] G. Melchers and H. Claes, Naturwissenschaften 31, 21/22. 249 (1943).

[4] L. P. Zhdanova, Doklady Akad. Nauk SSSR 70, 4, 715-718 (1950).

[5] G. Melchers and A. Lang, Naturwissenschaften Bd. 30, 588 (1942).

[6] S. Nakayama, Bot. Mag. (Tokyo), 68, 800, 61 (1955).

[7] B. Elliot and A. Leopold, Plant Physiol. 27, 4, 787 (1952).

[8] A. Leopold and F. Guernsey, Physiol. plantarum 7, 1, 29 (1954).

[9] W. James, Rev. Plant Physiol. 4, 59 (1953).

[10] D. M. Mikhlin and P. A. Kolesnikov, Biokhimiya 12, 452 (1947).

[11] O. A. Ron'zhina, Doklady Akad. Nauk SSSR 113, 2, 462 (1957). •

[12] J. Bassham, K. Shibata, K. Steenberg, J. Bourdon, and M. Calvin, J. Amer. Chem. Soc. 78, 16, 4120 (1956).

[13] R. Bidwell, G. Krotkov and G. Reed, Canad. J. Bot. 33, 189 (1955).

[14] O. B. Zalenskii, Botan. Zhurnal 42, 11, 1674 (1957).

[15] K. Thimann, C. Yocum and D. Hackett, Arch. Biochem. and Biophys. 53, 1, 239-258 (1954).

[16] G. Stenlid, Physiol. plantarum 2, 1, 61 (1949).

[17] B. A. Rubin, E. V. Artsikhovskaya and T. M. Ivanova, Collection: The Biochemistry of Fruits and Vegetables 2, (Izd. Akad. Nauk SSSR, 1951) [In Russian].

[18] O. A. Semikhatova, Trudy Komarov Botan. Institut, Akad. Nauk SSSR, Ser. IV, vol. 10, 296 (Izd. Akad. Nauk SSSR, 1955).

[19] V. James, Plant Respiration, chapter 9, (IL, 1956) [Russian translation].

[20] B. Chance and R. Sager, Plant physiol. 32, 6, 548 (1957).

[21] B. A. Rubin, I. A. Chernavina and A. V. Mikheeva, Doklady Akad. Nauk SSSR 105, 5, 1039 (1955).

[22] N. P. Voskresenskaya and E. G. Zak, Doklady Akad. Nauk SSSR 114, 2, 375 (1957). •

[23] L. Weinstein and W. Robbins, Contrib. Boyce Thompson Inst. 18, 225, 5 (1955).

[24] M. Kh. Chailakhyan and A. N. Boyarkin, Doklady Akad. Nauk SSSR 105, 3, 592 (1955).

[25] M. Kh. Chailakhyan, Fiziol. Rastenii 5, 541 (1958). •

[26] I. N. Borodina, Trudy po prikl. botan. genet. i selektsii 27, V. 5, 171 (1931).

[27] M. Kh. Chailakhyan, Doklady Akad. Nauk SSSR 48, 5, 382 (1945).

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• See English translation.

THE TRANSFORMATION OF SUCROSE IN THE FIBROVASCULAR BUNDLES OF SUGAR BEET

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In one of the earlier works of our laboratory it was shown that the fibrovascular bundles of sugar beet and plantain support intensive respiration, exceeding by several times that of the surrounding tissues [1]. The high intensity of respiration of the conducting bundles also was established for other species of plants [2, 3, 6]. This made it possible to assume that the conducting system of plants is characterized by energy metabolism.

Further study of the metabolism of the fibrovascular bundles would be of great interest in explaining the mechanism of the movement of nutrient substances in them, if the conducting system takes an active part in the realization of this function. Therefore, at the present time a great deal of attention is being paid to the study of the metabolism of the conducting tissues [2-9]. In connection with this, a whole series of works was devoted to the study of enzymes in the fibrovascular bundles. It was shown that respiration of the conducting bundles of sugar beet and other plants that were studied is differentiated from the respiration of the surrounding tissues by the prevalence of cytochromoxidase over other oxidases [2, 3, 4], which shows the singularity of the final stage of their respiration. Besides this, we observed active succinodehydrase in the conducting bundles of cow parsnip (Heracleum mantagazzianum), which gave the basis for the assumption that processes in these tissues proceed according to the di- and tricarbonic acid cycle [3]. Still earlier, active phosphatase and phosphorylase were observed in the fibrovascular bundles of sugar beet [5].

A more detailed study of the enzymes of the conducting tissues of sugar beet was carried out recently by Kursanov, Pavlinova, and Afanas'eva [9]. In this work the authors established the presence in the conducting bundles of sugar beet of a very active hexokinase that accomplishes phosphorylation of hexoses through ATP, an aldolase decomposing fructoso-1,6-diphosphate into two molecules of phosphotriose, and also phosphohexoisomerase and apirase. It is interesting to note that the activity of the hexokinase does not stop gradually over the entire vegetative period. Its greatest activity coincides with the time of intensive flow of sucrose from the leaf blades into the root. At the same time the activity of the hexokinase in the tissues of the parenchyma of the petiole that surround the fibrovascular bundles is not great and does not undergo any noticeable changes over the period of vegetation.

These data, although indirect, make it possible to suggest the presence of a connection between the metabolism of the conducting tissues and the movement of nutrient substances in them. Earlier a number of authors observed such a connection when inhibiting the conducting tissues with respiratory poisons, which usually led to a stop in the transport of assimilates [2].

This work is a continuation of the investigations on the metabolism of the conducting tissues which were started in our laboratory and is devoted to a study of the conversion of sucrose in the conducting tissues. Sucrose is the basic transport form in almost all studies of plants. Therefore, it seemed expedient to study the conversion of sucrose in the conducting tissues. Carbohydrates are the basic respiratory material of the conducting tissues, as the measurements of the respiration coefficient of the fibrovascular bundles showed [2, 6]. The discovery by Kursanov, Pavlinova, and Afanas'eva [9] of glycolytic enzymes in the conducting bundles of sugar beets gives the basis for assuming that the beginning stage of the respiratory conversion of carbohydrates takes place here

along a glycolytic path. The presence of glycose-6-phosphate and fructoso-6-phosphate in the conducting tissues [9] are also a confirmation of this. Therefore, we put before us the goal of studying the conversions of sucrose connected with the further conversions of this sugar in the process of respiration. For this we traced the formation of organic acids from the sucrose and their use in the synthesis of amino acids.

METHODS

Sugar beets of variety Verkhnyachok were used as subjects as in the earlier works of our laboratory. The conducting tissues were taken immediately before the experiment from the leaf petiole, cut into pieces 2 to 3 cm long and placed in a 0.05 M solution of marked sucrose with a specific radioactivity near 100,000 impulses per minute per ml. The experiments were carried out either in a Warburg instrument at 30° or in a thermostat at 25°.

TABLE 1

The Formation of Monosaccharides from C_{14}-Sucrose in the Conducting Bundles and Petioles of Sugar Beet (in impulses/minute for 1 g green weight of tissue; exposure, 2 hours at 30°)

Expt. No.	Tissues	Sum of all sugars	Monosaccharides				Percent of monosaccharides
			sucrose	glucose	fructose	Sum of monosaccharides	
1	Conducting bundles	21 708	21 235	295	178	473	2.2
2	Conducting bundles	14 947	14 737	210	0	210	1.4
	Parenchyma of the petioles (without conducting bundles)	1 732	1333	266	133	399	23

For the experiments in the Warburg instrument, the material was placed in the basic part of the container, and the sucrose was added from a lateral tap. In the middle container we placed 20% NaOH for absorption of the CO_2. After the end of the period of exposure (1-3 hours) the conducting tissues were washed 10 times with water, fixed with 96% alcohol and then doubly extracted with 80% ethanol. We measured the radioactivity in the extract, on the basis of which we judged the quantity of the absorbed sucrose. From the middle container of the Warburg container we quantitatively extracted the alkali and the carbon dioxide connected to it, which was precipitated in the form of $BaCO_3$. The $BaCO_3$ precipitate was washed with ethanol on centrifuging, and we then examined it for radioactivity.

The alcohol extract from the conducting bundles was analyzed for the concentration of sugars, organic acids and amino acids. For this, the alcohol was distilled off in a vacuum at 35°. The remains were dissolved in a small quantity of warm water. A small part of the water solution was used for measuring the sugars by the method of paper chromatography. For this, n-butanol, acetic acid, and water in the ratio 4:1:5 were used as the solvent. Resorcin phosphate served as a developer on the ketoses and aniline phthalate served on the aldoses [10].

We measured the sugars without a preliminary removal of amino acids and organic acids from the solutions because the concentration of sugars in the conducting bundles of the sugar beet is very high and for this, in order to observe them on the chromatogram, a quantity of the extract corresponding to not less than 10 mg of fresh weight of the material must be placed on the spot, just as for the observation of organic acids and amino acids not less than 300-500 mg of material is necessary. With this the radioactivity of the latter two in comparison with the sugars is so much less that it is not observed in the same dilution as is used in the material for the determination of sugars.

In order to separate the amino acids from the organic acids and sugars, a water solution of the conducting tissues is passed through the column of the strongly basic cation, Dowex 50. The amino acids are then eluted from the column with the help of 10% NH_4OH. Ammonia was removed by concentrating the eluate. The dry remains containing amino acids were dissolved in water and placed on chromatograms. For separation of the

amino acids, we used the solvents n-butanol, acetic acid., and water in the ratios 4 : 1 : 5 and 8 : 3 : 1. In several cases n-butanol, formic acid,and water in the ratio 90 : 10 : 45 were used as a solvent for the identification of individual amino acids. As a developer for the amino acids we used ninhydrin, or more often isatin, the use of which significantly improved the identification of amino acids [11].

Sugars and organic acids remained in the water solutions after passing through the cation.

For separation of the organic acids from the sugars, we concentrated a water solution until dry, and separated the fraction of the organic acids with the help of the widespread method based on the solubility of the organic acids in the acidified sulfur ester without water [12].

In general we used n-butanol, formic acid,and water in the ratio 95 : 10 : 20 and ethanol, ammonia,and water in the ratio 80 : 10 : 10 as a solvent in order to separate the organic acids on the chromatograms. Brom cresol purple served as the developer. Qualitative reactions were used for identification of the individual acids [10].

Excised fibrovascular bundles were fixed with liquid nitrogen for the determination of the ketoacids. Separation of the ketoacids was carried out using their hydrazones [13, 14, 15]. At first we used a solvent of n-butanol, ethanol,and water in the ratio 1 : 4 : 5 in order to divide the hydrazones of the ketoacids on the chromatograms. In this case the hydrazones of the ketoacids had similar levels, which made it possible to separate them from the fairly significant impurities. Then the lemon-yellow color of the hydrazones of the ketoacids was eluted from the chromatogram and again placed on paper previously treated with a phosphate buffer with pH 7.2. With the second distillation we used a solvent butanol, ethanol,and a phosphate buffer (pH 7.2) in the ratio 4 : 1 : 5. The identification of the hydrazones of the ketoacids was accomplished by matching spots with the corresponding standards and also by their color after sprinkling the chromatogram with an alcohol-soluble alkali [16]. Besides this, we carried out a transfer of the hydrazones of the ketoacids into the corresponding amino acids by the Virtanen method [17].

TABLE 2

This Use of the Labeled Sucrose in the Respiration of the Fibrovascular Bundles of Sugar Beet (in impulses/minute and mg per 1 g of green weight)

Experimental number	Exposure hours	Absorption of sucrose		$C^{14}O_2$ given off		
		impulses	mg	impulses per minute	mg of absorbed sucrose	% of absorbed sucrose
1	1	23 965	4.1	355	0.06	1.5
2	3	50 715	8.6	3465	0.59	6.8
3	3	38 242	6.5	2551	0.43	6.7

The separation of the sugars, organic acids,and amino acids was carried out on a single scale chromatogram at 18-20°. The paper used was Volodarsk Leningrad Factory No. 2. The undeveloped spots of the sugars, organic acids, or amino acids were eluted from the chromatogram with 50% alcohol, and we then determined the radioactivity of the separate substances with the help of a face meter. The figures for the radioactivity that are given in the article correspond to the data obtained directly from the meter. The efficiency of the calculation was 9%.

EXPERIMENTAL

The special experiments showed that when the fibrovascular bundles of sugar beet were held for a period of 1-3 hours in a 0.05 M solution of labeled sucrose, it was possible for the sugar to enter into the conducting tissues in significant quantities. This made it possible to follow the conversion of sucrose in the conducting bundles.

First of all, we turned our attention to the conversions that can take place in the composition in the conducting tissues of the sugars themselves. The possible conversions of sugars in the leaf petioles of sugar beet were

TABLE 3

The Formation of Radioactive Organic Acids in the Conducting Bundles of Sugar Beet from Labeled Sucrose (in impulses/minute per 1 g of green weight)

Acids	Experiment 1 (exposure 2 hours)	Experiment 2 (exposure 2.5 hours)
Place of application	34	96
Oxalic	132	–
Acid x	92	–
Citric	241	534
Malic	166	683
Glycolic	200	845
Lactic	–	51
Succinic	103	218
Fumaric	–	58
Total	876	2485
Radioactivity of the alcohol fraction	34 324	46 520
Percent included in the acid	2.4	5.3

already shown in 1933 in the work of Kursanov and Kazakova [18] in which the authors, in filtering glucose or fructose under pressure through cuttings of the leaf petioles, found a deficient form of hexose and sucrose in small quantities, in the filtrate. William [19] also showed the possibility of the synthesis of sucrose in the conducting bundles of sugar beet. In 1955 Pavlinova [6] observed that marked fructose moving in the conducting system of sugar beet is often converted into sucrose. Thus, in one of the experiments on the movement of C^{14}-fructose through the conducting system, already more than 10% of the radioactivity could be found in the sucrose after 15 minutes.

In our experiments, as already shown above, we held the conducting bundles of sugar beet in a 0.05 M solution of evenly labeled sucrose and, therefore, it was possible to trace the reverse reaction, that is, the conversion of sucrose into monosaccharides. The results of two of these experiments are presented in Table 1.

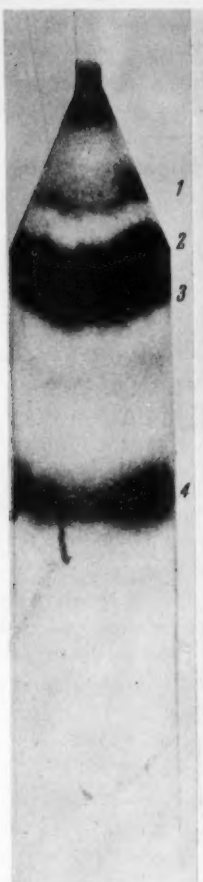
As we can see from Table 1, the formation of monosaccharides from sucrose takes place very slowly in the conducting tissues. This is particularly noticeable when these data are compared with the results obtained in similar experiments with the tissue of the parenchyma surrounding the fibrovascular bundles. Special experiments showed that the tissues of the petiole from which the conducting bundles were removed absorb sucrose from the external solution several times more slowly than the fibrovascular bundles. In connection with this, only a small quantity of the marked sucrose is able to enter into the tissues of the parenchyma of the petiole. Nevertheless, as we can see from Table 1, the sucrose introduced into the tissues of the petiole resulted after two hours in an inverse of 23%, while in the conducting bundles 1.4-2.2% of the absorbed sucrose was inverted after the same period of time. The data obtained are in conformity with the low activity of the invertase in the conducting bundles and the high activity of this enzyme in the parenchyma of the petiole [5]. Thus, we can conclude that in the conducting bundles, in contrast to the surrounding tissues, there are favorable conditions for the maintenance of sucrose in the noninverted condition. This probably has some significance for the mechanism of the transport of sucrose.

Nevertheless, the experiments carried out in the Warburg instrument showed that sucrose undergoes conversion in the conducting bundles as a result of which part of it shows an oxidation into CO_2 and H_2O (Table 2).

The results presented in Table 2 show that the fibrovascular bundles of sugar beet in the process of respiration oxidize 1.5% of the absorbed sucrose into CO_2 after 1 hour and more than 6% after 3 hours. These data agree well with the conclusion which we made earlier on the basis of the determination of the respiration coefficient that carbohydrates are the basic respiratory substrate of the conducting tissues of sugar beet [4]. As shown above, the presence in the fibrovascular bundles of active glycolytic enzymes gives the basis for the supposition that the beginning stage of the respiratory conversion of carbohydrates in these tissues has a glycolytic character. In the conducting bundles of sugar beet, neither riboso-5-phosphate nor sedoheptulose-7-phosphate were found. This apparently shows that direct oxidizing decomposition of glucose has no place in these tissues [9].

For this, in order to trace the conversion of the radioactive sucrose connected with its further stages of division, we made observations on the appearance of radioactive traces in the organic acids.

The central position occupied by pyruvic acid in the aerobic and anaerobic conversions of carbohydrates induced us to undertake first of all a search for pyruvate and other ketoacids in the fibrovascular bundles. For this, pyruvic acid, α-ketoglutaric acid, and glyoxalic acid were found as hydrazones. The transfer of the hydrazones of keto acids into the corresponding amino acids suggests the presence of pyruvic acid (in the form of alanine),



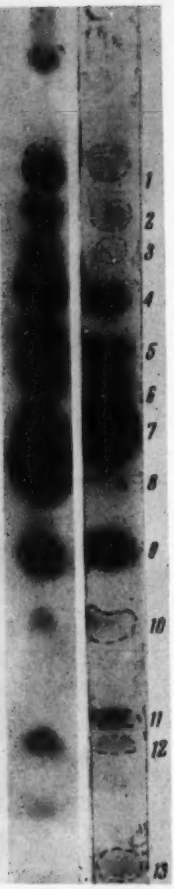


Fig. 1 Fig. 2 Fig. 3

Fig. 1. Ascending chromatogram of amino acids obtained by means of hydrogenation of hydrazones of ketoacids. 1) Aspartic acid; 2) glycine + + serine; 3) glutamic acid; 4) alanine. Solvent: n-butanol, acetic acid, and phosphate buffer (pH 6) in the ratio 4: 1: 5 (Boyarkin and Dmitrieva [21]).

Fig. 2. Chromatogram of the organic acids of the conducting bundles of sugar beet. a) Remains; b) alcohol extract. 1) Acids giving the color with naphthoresorcin; 2) oxalic; 3) phosphate + x; 4) citric; 5) malic; 6) glycolic; 7) lactic; 8) succinic; 9) fumaric. Solvent: n-butanol, formic acid, and water in the ratio 95: 10: 20.

Fig. 3. Chromatogram of amino acids of the conducting bundles of sugar beet. 1) Lysine; 2) asparagine; 3) arginine; 4) aspartic acid + glutamine; 5) serine; 6) glycine; 7) glutamic acid; 8) threonine; 9) alanine; 10) proline; 11) γ-aminobutyric acid; 12) valine; 13) phenylalanine (Standards at the left). Solvent: n-butanol, acetic acid, and water in the ratio 4: 1: 5 (one time) and 8: 3: 1 (four times). Each time the solvent was drained down towards the end of the chromatogram, which was dried, and the solvent applied again.

TABLE 4

The Formation of Radioactive Amino Acids in the Conducting Bundles of Sugar Beet from C^{14}-Sucrose (in impulses/minute per 1 g of green weight; exposure, 3 hours)

Amino acids	Experiment 1		Experiment 2		Amino acids	Experiment 1		Experiment 2	
	Imp/min	%	Imp/min	%		Imp/min	%	Imp/min	%
Lysine	32	1.8	22	2.5	Proline	46	2.7	10	1.2
Asparagine	97	5.5	74	8.6	γ-aminobutyric acid	212	12.2	105	12.5
Arginine	7	0.4	5	0.5	Valine	41	2.4	9	1.2
Aspartic acid	137	7.9	+ serine 90	10.4	Phenylalanine } Leucine }	31	1.7	4	0.2
Glutamine			23	2.6	Total	1744	100	862	100
Serine	94	5.4	–	–	Radioactivity of the alcohol fraction	47 250		35 691	
Glycine			13	1.5					
Glutamic acid	645	37	353	41					
Threonine	?	?	8	0.9					
Alanine	402	23	148	17.2	Percent included in amino acids	3.7		2.4	

α-ketoglutaric acid (in the form of glycine) (Fig. 1). Besides this, we can see from Fig. 1 that aspartic acid is found in the chromatogram, which shows the presence in the fibrovascular bundles also of oxaloacetic acid. It is interesting to note that Krupka and Towers [16] in shoots of wheat, and Towers and Mortimer [20] in the leaves of sugar beet, did not find oxaloacetic acid in the form of a hydrazone and could identify it only in the form of aspartic acid.

Finally, the use as a solvent of n-butanol, acetic acid, and water in the ratio 4: 1: 5 (one time) and 8: 3: 1 (four times) made it possible to separate from the spot of glycine that of serine, which showed the presence in the fibrovascular bundles also of oxypyruvic acid. Apart from this, other spots were observed in the area of lysine, threonine, proline, and leucine, although in very small quantities.

The presence of pyruvic acid in the fibrovascular bundles of sugar beets agrees well with the presence in these tissues of very active aldolase separating fructoso-1, 6-diphosphate into 3-phosphoglycerine aldehyde and phosphodioxyacetone [9]. As is well known, the further conversion of these trioses by means of glycolysis also leads to the formation of pyruvic acid.

Together with this, the presence in the fibrovascular bundles of sugar beet, of oxaloacetic acid and α-ketoglutaric acid besides the pyruvic acid show the functioning in these tissues of the di- and tricarbonic acid cycle. Actually, as we can see in Fig. 2, b, citric and malic acids were found in the fibrovascular bundles in significant quantities and also succinic and fumaric acids in perceptible quantities. Fumaric acid is readily seen on the chromatogram in the form of a dark spot under ultraviolet [10]. In this same material was also found a small quantity of lactic acid which suggests the presence of glycolysis in the conducting tissues. Besides this, as we see from Fig. 2b, glycolic, oxalic and an unidentified acid (x) were found. This acid occupies the place of tartaric acid on the chromatogram; however, it does not give a positive reaction with ammonium vanadate [10]. Inorganic phosphorus was observed in this same spot. Finally, a spot giving a positive reaction on the uronic acids with naphthoresorcin but not galacturonic acid, was found in the place of application.

Thus, the presence in the fibrovascular bundles, along with pyruvic acid, of such acids as oxaloacetic, α-ketoglutaric, malic, citric, succinic and fumaric makes it probable that the Krebs cycle functions very actively in these tissues. However, on the strength of the specific function of the conducting tissues, only one constatation in them of these or other substances still does not make it possible to assume that the compounds that were found were formed in the fibrovascular bundles themselves. On the contrary, the presence of these or other substances in the conducting system is normally connected with their transport. The method of trace atoms makes it possible to isolate the group of mobile substances, especially in those cases when the analysis of the conducting tissues

proceeds in the immediate minutes after the assimilation into them of the radioactive products, as this had a place in the experiments of Kursanov, Brovchenko and Parliskaya [22].

Our experiments were carried out with the isolated fibrovascular bundles, in which only labeled sucrose could enter; therefore, the development of the traces of these or other compounds with certainty could be shown on their formation from the sucrose in the conducting bundles themselves.

Actually, radioactive keto acids and other organic acids are formed in the fibrovascuair bundles of sugar beet when they are held in a solution of marked sucrose. The distribution of the radioactivity in the organic acid is presented in Table 3.

As we can see from Table 3, after 2-2.5 hours a radioactive trace is developed in all of the organic acids transferred into the alcohol fraction on use by the conducting bundles of sugar beet of radioactive sucrose. This gives a basis for supposing that the bundles of the sugar beet themselves are capable of synthesizing all of the organic acids found in them. With this, the development of the mark in the citric, malic, succinic, and fumaric acids is shown in the fact that the process of oxidation of the molecule of the sugar proceeds here through the di- and tricarboxylic acid cycle. In the second experiment, attention is turned to the significant radioactivity of the succinic acid at the time that its absolute concentration in the conducting bundles is not high (see Fig. 2). This is possibly connected with the activity of the succindehydrase, which was found by Ziegler [3] in the tissues of the phloem of Herculeum mantegazzianum and which takes part in the further conversions of the succinic acid.

We can see when looking at Table 3 that the lactic acid is also shown to be marked, although fairly weakly, which again shows the glycolysis proceeding in these tissues. Together with this, we can again see from the same table that sucrose is used in the conducting bundles also in the synthesis of acids not participating in the Krebs cycle, glycolic and oxalic. Especially high radioactivity is observed in the glycolic acid, which in the second experiment even exceeds the activity of the citric and malic acids.

The data obtained make it possible to suppose that other means for the enzyme conversion of this sugar exist in the conducting bundles, along with the oxidation of sucrose in the process of respiration along the path of glycolysis and the Krebs cycle.

Link, Klein and Barron [23] observed an increase in the concentration of glycolic acid in the stems of tomatoes while incubating them in anaerobic conditions with malic acid. The authors suggested the presence in normal conditions in these tissues of the following chain reaction: malic acid $\xrightleftharpoons{+H_2O}$ 2 glycolic acid $\xrightleftharpoons{-2H_2}$ 2 glyoxalic acid $\xrightleftharpoons{+O_2-2CO_2}$ formic acid $\xrightarrow{-H_2}$ oxalic acid. In support of this scheme, they found a very active dehydrase of formic acid and a high concentration of oxalic acid in the tomato stems.

The accumulation of radioactivity in our experiments in the malic and glycolic acid, and also the development of a mark in the oxalic acid, makes it probable that similar conversions have a place in the fibrovascular bundles of sugar beet. Glyoxalic acid is also present in the fibrovascular bundles to the extent that this was shown above.

In the literature there are indications of several possible paths for the synthesis of glyoxalic acid [20, 24, 25, 26]. However, the presence in the conducting bundles, along with the glyoxalic acid, of glycolic and oxalic acids makes all the more probable the formation of glyoxalic acid from the glycolic acid on the path toward the synthesis of oxalic acid. As we can see in Fig. 2a, the concentration of oxalic acid in the conducting bundles is sufficiently high. Its basic mass here is in the form of apparently insoluble potassium salts and, therefore, it does not move into the alcohol extract. The determination of oxalic acid in this case was carried out after treatment of the dry precipitate with 25% H_2SO_4.

In order to explain to what degree the formation of oxalic acid is characteristic just for the conducting system, we undertook the determination of organic acids in the parenchyma tissues of the petiole surrounding the fibrovascular bundles. The experiments showed that the petioles of sugar beet contain an even greater quantity of oxalic and glycolic acids. Glyoxalic acid was also found in them. All of these acids are labeled with C^{14} when the tissues of the petiole (without the conducting bundles) are held in a solution of marked sucrose, as in the conducting tissues. Thus, the synthesis of oxalic acid is not an abnormal feature of the metabolism of the conducting bundles.

The labeled sucrose carried into the fibrovascular bundles can also be used in the synthesis of amino acids.

The following amino acids were found in the conducting tissues of sugar beet (Fig. 3): traces of lysine, arginine, asparagine, aspartic acid, glutamine, serine, traces of glycine, glutamic acid, traces of threonine, alanine, proline, γ-aminobutyric acid, valine, phenylalanine, and leucine.

Quantitatively, glutamic acid and alanine predominated significantly above all of the remaining amino acids. The data on the determination of the radioactivity in the separate amino acids when the conducting bundles are incubated with C_{14}-sucrose are presented in Table 4.

The data of Table 4 show that sucrose is actually used in the synthesis of amino acids in the conducting bundles. With this, glutamic acid and alanine are especially intensively brought forth. There is a great deal of data in the literature, obtained both for plant and in animal objects, that also show the predominant formation of these acids, especially glutamic acid, from sugars [26, 27, 28].

A high concentration in the fibrovascular bundles of sugar beet of α-ketoglutaric and pyruvic acids (Fig. 1), the presence of a significant quantity of glutamic acid and alanine (Fig. 3) formed on the base of these ketoacids and, finally, the rapid inclusion of labels into these amino acids when sucrose is used show that this group of compounds occupies an important place in the metabolism of the conducting tissues.

We can also see from Table 4 that the significant radioactivity is centered also in the γ-aminobutyric acid, the conversion of which is closely connected with glutamic acid.

In this manner our experiments show that sucrose undergoes various conversions in the fibrovascular bundles of sugar beet. It follows from this that sucrose moving in the conducting system of beet (at a rate close to 100 cm/hour) often can be used in the metabolism of the conducting tissues themselves. Considering the high concentration of sugars in the fibrovascular bundles of sugar beet and the great rate of their movement, it can be assumed that the consumption of sucrose in these conversions is not high in comparison with the normal quantity of sugar moving through at the same time. Nevertheless, in these processes a whole series of intermediate and secondary substances is formed which can be accumulated gradually. Therefore, great care is required in appraising the progress of substances found in the conducting system. It is necessary to differentiate strongly the naturally transported compounds from the substances originating secondarily as a result of the metabolism of the conducting tissues themselves.

The basic path of the conversion of sucrose in the conducting bundles of sugar beet takes place through glycolysis and the Krebs cycle, which must be accompanied by an accumulation in them of energy in the form of high-energy bonds. However, up to the present time we still do not know of concrete connections between the transport of sucrose and the use of energy for its accomplishment. The explanation of these connections is necessary, although it is also a very difficult task. We hope to turn to its study in the near future.

SUMMARY

1. The introduction of evenly labeled sucrose (C_{14}) into isolated fibrovascular bundles of sugar beet showed that sucrose can undergo various conversions in these tissues.

The basic path of these conversions is in the use of sucrose in the conducting bundles as respiratory material, which leads to the formation of a number of intermediate products and to the emission of radioactive CO_2.

2. Among the intermediate products of the decomposition of sucrose, the following organic acids were identified: citric, malic, succinic, fumaric (Fig. 2) and also α-ketoglutaric, pyruvic, oxypyruvic and oxaloacetic acids (Fig. 1), which shows the functioning in the conducting bundles of the di- and tricarboxylic acid cycle. Besides this, significant quantities of glycolic, glyoxalic and oxalic acids are formed in the fibrovascular bundles of sugar beet. This makes it probable that in the conducting bundles secondary oxidizing conversions of sucrose takes place on a base of malic acid through glycolic, glyoxalic and oxalic acids.

3. A part of the organic acids is used by the fibrovascular bundles in the synthesis of various amino acids, among which the following were identified: lysine, arginine, asparagine, aspartic acid, glutanine, serine, glycine, glutamic acid, alanine, threonine, proline, γ-aminobutyric acid, valine, phenylalanine and leucine.

The inclusion of the labeled C_{14} in glutamic acid and alanine proceeds especially rapidly, which also corresponds to their high normal concentration in the conducting bundles of sugar beet. The presence of α-ketoglutaric

and pyruvic acids makes it probable that glutamic acid and alanine develop here as a result of amination and reamination of the indicated ketoacids.

4. The division of the sucrose by invertase into monosaccharides is accomplished slowly in the living tissues of the fibrovascular bundles (see Table 1). This supports our first conclusions concerning the low activity of invertase in the conducting tissues.

5. The data obtained make it possible to suppose that part of the sucrose included in the fibrovascular bundles is used in respiration. This process probably is not great in comparison with the normal mass of the sucrose transported through the conducting tissues. Nevertheless, sufficient quantity of high-energy bonds necessary for maintaining the active life of the fibrovascular bundles and, possibly, for fulfilling the work connected with the transport of organic compounds can be created in the process of respiration.

6. When studying the composition of the moving organic substances with the help of the method of tracer atoms, it is necessary to consider the possibility of their secondary conversions in the conducting tissues. For this, shorter exposures, in which the secondary conversions will be held to a minimum, are desirable.

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LITERATURE CITED

[1] A. L. Kursanov and M. V. Turkina, Doklady Akad. Nauk SSSR 84, 5, 1073 (1952).

[2] I. Willenbrink, Planta 48, 269 (1957).

[3] H. Ziegler, Planta 51, 186 (1958).

[4] M. V. Turkina and I. M. Dubinina, Doklady Akad. Nauk SSSR 95, 1, 199 (1954).

[5] A. L. Kursanov and M. V. Turkina, Doklady Akad. Nauk SSSR 95, 4, 885 (1954).

[6] O. A. Pavlinova, Fiziol. Rastenii 2, 378 (1955).

[7] H. Wanner, Ber Schweiz. bot. Ges. 63, 201 (1953).

[8] Tsao Tsung-hsum and Lin Chin-ju, Acta bot. sinica 6, 269 (1957).

[9] A. L. Kursanov, O. A. Pavlinova, and T. P. Afanas'eva, Fiziol. Rastenii 6, 286 (1959).*

[10] K. Paech and M. Tracey, Modern Methods of Plant Analysis, B. v. 11 (1955).

[11] A. N. Boyarkin, Fiziol. Rastenii 5, 86 (1958).*

[12] G. W. Pucher, A. I. Wakeman, and H. B. Vickery, Industr. and Engng. chem. Analyt. Ed. 1, 244 (1941).

[13] Kun and Hernander, Biochem. et biophys. acta 23, 181 (1957).

[14] D. Cavallini and N. Frontali, Biochim. et biophys. acta 13, 439 (1954).

[15] A. Virtanen and V. Miettiinen, Acta Chem. Scand. 7, 38 (1953).

[16] K. M. Krupka and G. H. N. Towers, Canad. J. Bot. 36, 165 (1958).

[17] A. Virtanen and M. Aeffhol, Acta chem. Scan. 8, 1720 (1954).

[18] A. L. Kursanov and M. N. Kazakova, Trudy TsINS, I, V 12 (1933).

[19] A. William, Arch. Inst. bot. Univ. Léige (Belgique), 18, 1 (1948).

[20] G. H. Towers and D. C. Mortimer, Canad. J. Biochem. and Physiol. 34, 511 (1956).

[21] A. N. Boyarkin and M. I. Dimitrieva, Fiziol. Rastenii 5, 386 (1958).*

[22] A. L. Kursanov, M. I. Brovchenko, and A. N. Parfiskaya, Fiziol. Rastenii 6, 527 (1959).*

[23] G. K. K. Link, R. M. Klein, and E. S. Guzman Barron, J. Expti. Bot. 3, 216 (1952).

* See English translation.

[24] R. M. Krupka and G. H. N. Towers, Canad. J. Bot. 36, 179 (1958).

[25] H. L. Kornberg and H. A. Krebs, Nature 179, 4568, 988 (1957).

[26] B. I. Rogers, Plant Physiol. 30, 377 (1955),

[27] É. I. Vyskrebentseva, Biokhimya 22, 657 (1957).

[28] Vasuro Tsukad, Vutaka Nacata, Shusuke Hirano and Genkichiro Takagaki, J. Biochem. 45, 979 (1958).

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BRIEF COMMUNICATIONS

ENZYMATIC REDUCTION OF CARBON DIOXIDE

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Isolation of an enzyme which effects reduction of carbon dioxide in plants is very important in understanding the chemistry of the entire process of carbon assimilation and opens possibilities of reproducing highly important syntheses in vitro. At one time it was assumed that pyridine nucleotides were capable of such reduction. However, the negligible carbon dioxide assimilation in their presence [1], and further, the necessity of adding the whole leaf extract [2] makes this assumption doubtful. Recently an enzyme was isolated from plants without which it is impossible to reduce either the pyridine nucleotides themselves or the carbon dioxide. It was named photosynthetic reductase [3]. Judging from the method of its isolation from the leaves and its properties, this enzyme resembles one earlier isolated by us from various groups of green plants and named hydrogenase. In cells it is found within the same fraction of substances where the primary photosynthesis products are located [4, 5]. For isolating the hydrogenase enzyme, leaves of white clover or primrose were cut while chilled in an 0.6 M sucrose solution, the extract filtered through linen and a glass filter and precipitated with 60% acetone. Saccharides and other substances were washed out with water from the acetone precipitate, after which an insoluble complex remained. This amounted to nearly 10% dry weight in clover and nearly 6% in primrose. Experiments in carbon dioxide assimilation in darkness were conducted with this complex containing active hydrogenase and only flavins as pigments. They were conducted in Barcroft manometers in an atmosphere of hydrogen, nitrogen or air with addition of 3% gaseous CO_2, labeled with C^{14} (0.08% $C^{14}O_2$). The complex was first held cold in an atmosphere with labeled carbon dioxide to form an unstable combination with it. The experiment was then conducted for one hour at 20°. At the end of the hour the preparation was fixated at a temperature of solid carbon dioxide or liquid nitrogen and tested for its C^{14} content. The quantity of the complex in each experiment was 100 mg; the experiments were repeated over 50 times. Data given in Tables 1 and 2 were obtained with complexes from white clover leaves.

It is interesting to compare the maximum quantities of carbon dioxide assimilated by the complex with its Fe percentage. In complexes placed in darkness in air, the ferrous iron is usually almost 40% of the total; while in a hydrogen atmosphere, as well as in leaves during photosynthesis, this precentage increases. Possibly the increase in carbon dioxide reduction is related to this. Carbon dioxide assimilation up to 1% of the complex weight comprises 0.1% when calculated for the whole leaves, i. e., it equals the total fixation by plants in darkness.

TABLE 1

Complex Composition (in %) and Carbon Dioxide Assimilation

N	Fe	Mn	P	CO_2 assimilation, in % by weight		
				in air	in N_2	in H_2
5.7	1.1	0.1	0.6	0.37	0.67	1.08

When products of carbon dioxide assimilation were separated from the complex at 1-3° by trichloroacetic acid the major portion of C^{14} was again liberated in the form of carbon dioxide. Even when dissolved in water at the same temperature, almost half the C^{14} fixed by the complex was liberated as unstable carbon dioxide (Table 2).

The presence of unstably combined carbon dioxide in leaves has been mentioned in the literature in the past. Usually it is considered as the first step in the carbon path in photosynthesis [6, 7, 8].

An aqueous extract was precipitated by barium from 60% acetone, and C^{14} was found in the same polycarboxylic acid which is the initial photosynthetic product in whole leaves [5]. It contained iron and phosphorus, but not nitrogen. Carbon was found in the hydroxyl and carboxyl groups. In weight it consisted of nearly 1/6 of the complex. Almost all of the C^{14} was found in it after the unstable carbon dioxide was removed. A small amount of C^{14} always remained in the complex, perhaps in the products of polycarboxyacid decomposition. The value of polycarboxyacids as primary photosynthesis products in plants was established by Ruben [9] and later confirmed by many investigators [5, 10, 11]. Generation of the same products in experiments outside the cell by enzymatic assimilation of carbon dioxide serves as a new proof of this hypothesis.

To clarify whether only carbon dioxide fixation occurred in carboxyl groups, or whether reduction also occurred, decarboxylation was conducted by Ruben's method [9, 5]. It was found that only a portion of C^{14} was present in the carboxyl groups. The rest of the labeled carbon was found in the alcohol groups as shown by benzoylation according to the Schotten-Bauman method and subsequent decarboxylation of the products. Thus, we accomplished outside of the plant the reaction of Ruben and Kamen: carboxylation of carbon dioxide in the polycarboxyacid molecules and the reduction of a portion of carboxyl groups into alcohol groups at the same time.

TABLE 2

Assimilation Products of Carbon Dioxide in the Complex

Substance	Total percentage of C^{14}	Percentage of C^{14} in COOH	Percentage of C^{14} in COOH	Br number	Total percentage of C^{14} after Br
Undivided complex	100.0	83.6	16.5	156	55.3
Unstable CO_2	45.6	–	–	–	–
Polycarboxyacid	51.9	36.5	15.8	76	46.5
Remainder of complex	2.5	1.2	1.2	37	2.4

Polycarboxyacids isolated from the leaves after photosynthesis contained double bonds [5]. For polycarboxyacids obtained after the experiments outside of plants bromine numbers were determined by the Wild [12] micromethod. They were found to be high for the complex as a whole as well as for the polycarboxyacid.

At the same time it was found that on bromine addition exactly that C^{14} percentage was lost which corresponds to the unstable carbon dioxide. Warburg [7] showed that the unstable carbon dioxide from seaweed cells was removed by treatment with a NaF solution. According to our data, a reservoir containing comparatively large quantities of unstable carbon dioxide in cells is a complex possessing the highest bromine numbers in plants.

Related to this, evidently, is also its instability, as shown chromatographically (see figure). Complete decomposition occurred in a mixture of butyl alcohol with acetic acid. Partial decomposition was observed also without the acid, in butyl alcohol with water. But still a bright band of polycarboxyacid was seen here in the middle of the chromatogram. Better results were obtained in a mixture of butyl alcohol with ammonia; here practically the total radioactivity was found in the lower spot of a slowly advancing complex and in the band which separated the polycarboxyacid from it. These data resemble those obtained by Metzner [8] for primary products isolated from seaweed cells.

Decomposition with formation of various substances in polycarboxyacid isolated from whole leaves was described by us earlier [5]. The same results were observed on chromatography after bromination of polycarboxyacid obtained in experiments in vitro (see figure). The double bonds found in the primary products of carbon dioxide

reduction may facilitate its conversion in different directions [13], which confirms the hypothesis of early parting of synthetic paths in plants, already at the stage of this product, with a portion of the labeled carbon entering into complex protein substances [14, 15].

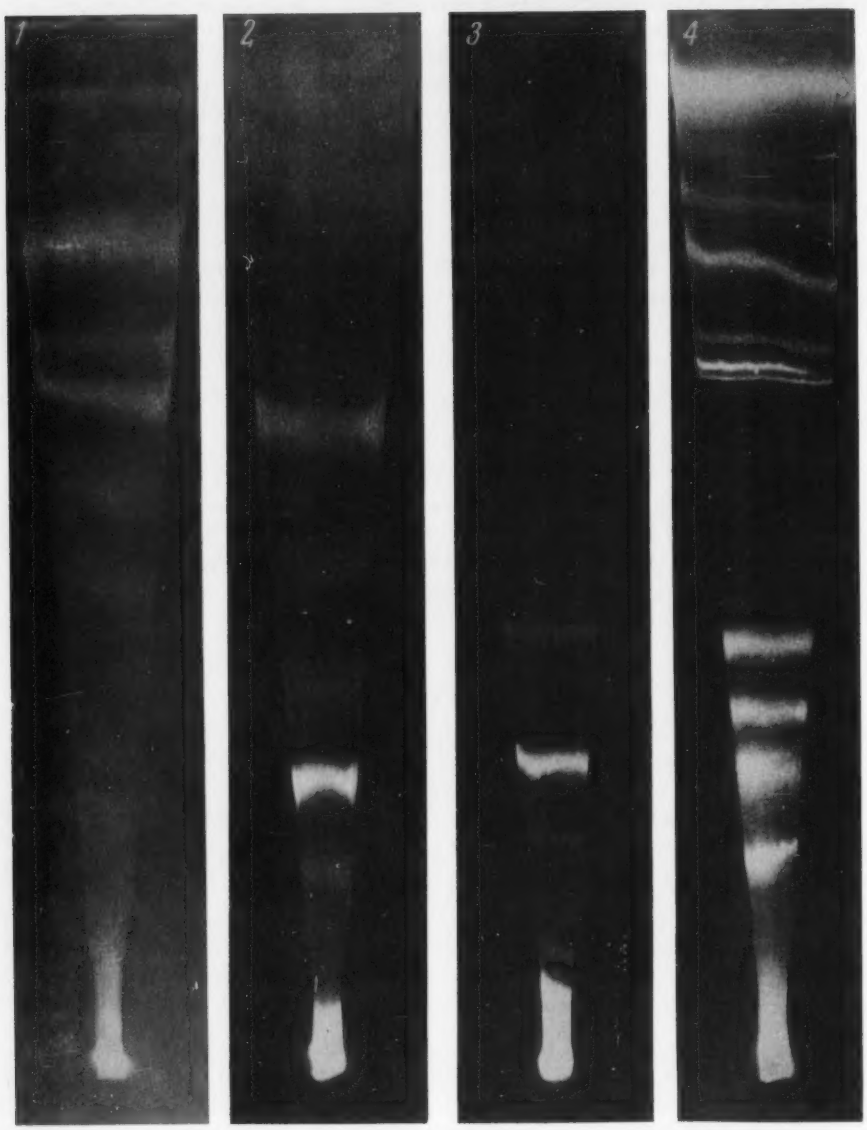

Figure. Autographs from radiochromatograms. 1) Extraction with 10% trichloroacetic acid (chromatography in a mixture of butyl alcohol, acetic acid and water in proportions of 4 : 1 : 5); 2) extraction by water (chromatography in butyl alcohol mixed with 1.5 N NH_4OH in proportion of 1 : 1); 4) extraction by water after bromination (chromatography in butyl alcohol mixed with water 1 : 1).

LITERATURE CITED

[1] W. Vishniac, R. Horecker and S. Ochoa, Advances Enzymol. 19, 1 (1957).

[2] A. Trebst, H. Tsujimoto, and D. Arnon, Nature 182, 351 (1958).

[3] A. San Pietro and H. Lang, J. Biol. Chem. 231, 211 (1958).

[4] O. Boichenko and M. Zakharova, Fiziol. Rastenii 1, 57 (1954); 6, 88 (1959).*

[5] O. Boichenko and M. Zakharova, Biokhimiya 21, 374 and 623 (1956).

[6] N. Doman, Biokhimiya 21, 78 (1956).

[7] O. Warburg, W. Schröder, and G. Krippahl, Angew. Chemie 69, 627 (1957).

[8] H. Metzner, Biol. Zbl. 77, 513 (1958).

[9] S. Ruben, M. Kamen, and W. Hassid, J. Amer. Chem. Soc. 62, 3443 (1940).

[10] A. Kuzin and G. Saenko, Biofizika 2, 213 (1957).

[11] O. Z. Kandler, Naturforsch, a, 12 b, 271 (1957).

[12] F. Wild, Estimation of Organic Compounds (Cambridge, 1953).

[13] V. Tauson, Izv. Akad. Nauk SSSR, Ser. Biol. 3, 423 (1947).

[14] A. Nichiporovich, Light and Carbon Nutrition in Plants [In Russian] (Izd. An SSSR, 1955).

[15] L. Nezgovorova, Fiziol. Rastenii 3, 497 (1956).

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* See English translation.

RINGING OF SHOOTS ON MATERNAL PLANTS AS A METHOD OF THEIR PROPAGATION

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The method of prior ringing of shoots on maternal plants is valuable for facilitating root formation in plants which form roots with difficulty. This method has been applied in horticulture for a long time to accelerate fruit bearing and to increase quantity and size of fruit. The ringing method promotes root formation due to accumulation above the ring of plastic substances flowing from the leaves. Studies by one of the authors [1] have shown that auxins are also retained above the ringing belt.

The original method of preliminary preparation of apple and pear tree shoots for grafting on a maternal plant by means of ringing was developed by Michurin [2]. But the method was not applied because it proved to be very cumbersome and laborious.

Broader prospects appeared for using a method of aerial cuttings on open soil related to the appearance of plastic fabrics impervious to water [3, 4, 5]. The use of polyethylene, vinyl and other fabrics in the ringing of shoots helped considerably in facilitating matters and reducing time for root formation. The operation consists in covering the ringed branches with a moistened moss saturated with a growth stimulant and wrapping it with the fabric. The growth stimulant can also be applied to the ringed spot in powder form. By retaining the moisture and conserving heat, the fabric greatly aids root formation.

Despite the long-established method of ringing, the question of this method's effectiveness as related to the shoot's age was completely neglected. It was also interesting to investigate the method of using polyethylene fabric in ringing tree shoots on open grounds. The latter as a rule are more difficult to graft than hothouse plants.

Experiments of preliminary preparation of shoots for grafting by ringing and the use of polyethylene fabrics were conducted for several years, chiefly on fruit-berry plants of the Plant Physiology Institute, on The Lenin Hills, Moscow, on an experimental plot.

The ringing was performed by removing a strip of bark, 0.3-0.5 cm wide around the shoot. Three varieties of apple trees were used: Antonovka, Shtreifling and Raika and cherry varieties Lyubskaya and Vladimirskaya. Experiments were conducted on shoots of different physiological conditions at 4 periods. The first ringing was performed during the second half of April on shoots of the past year's growth, when the buds were already swollen, but leaves had not as yet opened. By the middle of May shoots of the past year's growth with open leaves were ringed. On June 20-22 the slightly lignified green shoots were ringed, and, finally, at the end of July the fully lignified shoots of the current year's growth were ringed. The ringed spots were wrapped with moist peat-moss and above the moss a polyethylene fabric was applied. In one-half of the ringed shoots the moss was moistened by an IMK solution (10-20 mg/l) or by the potassium salt of heteroauxin (150-200 mg/l).

The moss remained moist in the polyethylene bag until the end of the experiment despite the dryness and high temperature of the surrounding air, so that there was no need for frequent moistening of the moss. The constant humidity of the moss aided callus formation on grafts of three ringing periods. All the ring shoots of the last

year's growth with opened leaves and the shoots of the current year's growth, whether lignified or weakly lignified, formed calluses while on the maternal plant, and individual weakly lignified shoots of Raika variety apple tree produced roots (Fig. 1, A). The callus formation was most rapid on green weakly lignified shoots of the current year's growth. But the ringed shoots of the previous year's growth with unopened leaves did not in most cases form calluses, and withered (85%). These experiments show very clearly that it is the leaves which create plastic substances directed downward to the ringed spots; without leaves the ringed shoots are deprived of these assimilants and die through emaciation.

Fig. 1. Ringed shoots of fruit trees forming roots on maternal plants. 2, A) Apple tree variety Raika; b) King orange variety Unshiu; C) Orange tree; on left, Mestnyi variety; on the right, Washington Navel variety.

A treatment with a growth stimulating solution accelerated callus formation. Thus, for instance, shoots of Antonovka apple tree and Lyubskaya cherry ringed on July 20 formed calluses around the whole shoot; in Lyubskaya by August 9, and in Antonovka by August 20 the calluses were of considerable size, while on the untreated shoots calluses at that time were weakly developed and formed only on one side of the base of the shoot.

After calluses formed the ringed shoots were cut off and were planted in earthenware jars, buried in an exposed soil seedbed. Further observations have shown that roots form best of all (in 20-25%) on grafts with calluses taken from shoots of the current year's growth and ringed at a weakly lignified state. These shoots had a lignified appearance by the time they were cut. Root formation in cut ringed shoots occurs partly during the current year and partly in the spring of the following year.

Callus formation can be obtained on green apple-tree grafts also without preliminary preparation of shoots on maternal plants. In the experiments of the first author [6], conducted together with V. I. Yegorova in 1945, a

good callus formation was successfully obtained on green grafts from apple tree varieties Antonovka, Korichnoe and Shtreifling even without ringing. But the green grafts with white tender calluses formed no roots and gradually died. The inability of these grafts to form roots is explained by their emaciation since all the nutrient substances were spent on the callus formation, which rapidly decays. But the graftings from the ringed shoots, when planted for rooting, are already in a lignified state with considerable reserves of nutrient substances, with well-formed calluses and therefore they can exist for a prolonged period without roots.

TABLE 1

Effect of Ringing on the Callus, and Root-Formation on Shoots of Subtropical Plants Not Separated from the Maternal Plants

Name of plant	Number of ringed shoots	Percentage of shoots	
		with calluses	with roots
King orange	35	37	5
Orange	40	37	27
Feijoa	40	50	5
Magnolia watsoni	20	75	15
Lithocarpus edulus	20	100	–
Kalmia angustifolia	26	42	–
Illicium floridanum	15	40	–

Indications that grafts with young porous white calluses take root much more poorly than grafts with yellow calluses formed for a longer period were found by Klyutsko [7] who experimented with woody Paradisca grafts.

Our experiments indicate that the callus in the ringed shoots plays a decisive role in root formation, since in these shoots it is the only organ having meristematic tissues, while the grafts cut from ringed shoots without calluses do not form roots.

Repeatedly checked results on fruit-berry plants of the temperate zone were used to conduct wider experiments in 1958 with subtropical fruit and decorative plants in the Batum Garden. As fruits, the Unshiu variety of king oranges, the Washington Navel and Mestnyi oranges and Feijoa were used; as decorative plants Magnolia watsoni, Lithocarpus edulus, Kalmia angustifolia L. and Illicium floridanum were used. Experimental results are reported in Table 1 for 2-3 months after ringing of shoots on the maternal plants.

As seen from the table, the ringing with the use of polyethylene material which preserves a moist atmosphere around the ringed spot for 2-3 months causes formation of calluses on the shoots and on some of them also, roots, even before separation from the maternal plant (Fig. 1, B and C). Only on rhododendron shoots neither calluses nor roots were formed.

The reported data show that a preliminary preparation of shoots on the maternal plant by ringing and applying of a growth stimulant changesthe physiological state of grafts in the direction of accelerating subsequent root formation. The method of ringing with use of a polyethylene fabric and moist moss soaked in a growth stimulant shows prospects of reproducing plants which form roots with difficulty in hothouse environments as well as in open soil.

LITERATURE CITED

[1] R. Kh. Turetskaya, Effect of Growth Substances on Root Formation as Related to Growth and Physiological Condition of Cuttings, Diss., Inst. Fiziol. Rastenii, AN SSSR (1944) [In Russian].

[2] I. V. Michurin, Collected Works, Vol. 1, Sel'khozgiz (1939) [In Russian].

[3] D. Wyman, Arnoldia 11, 718, 49 (1951).

[4] R. David, Compt. rend. Acad. Sci. 237, 19, 1175 (1953).

[5] J. L. Greech, Plants and Gardens 8, 298 (1952-1953).

[6] R. Kh. Turetskaya, Methods of Accelerating Plant Development by Grafting [In Russian], Izd. AN SSSR (1949).

[7] N. N. Klyutsko, Sel'skoe Khoz-vo Uzbekistana 9, 85 (1958).

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ACCUMULATION OF ASCORBIGEN IN PLANTS

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It has been established by many authors' studies that along with free ascorbic acid in plants there also is a combined form: ascorbigen [1,7]. Matusis [1] claims a higher vitamin value for ascorbigen than for ascorbic acid. On the other hand Sumtsov [2] indicates that ascorbigen shows a lower antiscorbutic activity, approximately $\frac{1}{2}$ to $\frac{1}{3}$ that of free ascorbic acid. At the same time Sumtsov [3] points out that ascorbigen is the most stable form of vitamin C.

At present it is known that ascorbigen is a complex, component parts of which are ascorbic acid and indolacetic acid [8].

A great number of studies have been devoted to investigations of ascorbic acid biosynthesis in plants, but little attention has been given to the study of ascorbigen. Factors affecting the formation of one or another form of ascorbic acid are yet unknown.

TABLE 1

Content of Total, Free and Combined Ascorbic Acid in Lettuce (mg %)

Forms of ascorbic acid	Control (water)	Ascorbic acid	Indolacetic acid	Indolacetic acid plus ascorbic acid	Forms of ascorbic acid	Control (water)	Ascorbic acid	Indolacetic acid	Indolacetic acid plus ascorbic acid
	After soaking seeds					After spraying plants			
Total	27.4	33.6	29.6	33.6	Total	28.7	46.7	32.5	38.4
Free	26.3	30.8	24.8	29.8	Free	28.3	44.6	29.2	36.1
Combined	1.2	2.8	4.8	3.8	Combined	0.4	2.1	3.3	2.3

Considering the above, we decided on a number of experiments to study the accumulation of ascorbigen and ascorbic acid in plants by a presowing treatment of seeds with solutions of indolacetic acid, ascorbic acid and a mixture of these components, and by spraying sprouts with these solutions. Plants with a short vegetative period were taken for these experiments: Icicle variety radish and lettuce.

The lettuce and radish seeds were soaked for 24 hours in these colutions: 1) ascorbic acid, 2) indolacetic acid, 3) a mixture of ascorbic and indolacetic acid, 4) control—water. The concentration of the solutions was 10 mg/l. Determinations of ascorbigen and ascorbic acid content were conducted on lettuce leaves on the 18th day, and on leaves and edible roots of radish the 20th day after sowing.

As seen from data in Table 1, the largest quantity of ascorbic acid was observed when lettuce seeds were treated by ascorbic acid and ascorbic acid plus indolacetic acid. As far as the combined form is concerned, its highest content is observed in treating the seeds with indolacetic acid alone; in the other two variants of the experiment it is also larger than in the control.

TABLE 2

Content of Total, Free and Combined Ascorbic Acid in Radish Roots and Leaves (mg %)

Ascorbic acid form	Content in radish edible roots				Content in radish leaves			
	control (water)	ascorbic acid	indol-acetic acid	indol-acetic acid plus ascorbic acid	control (water)	ascorbic acid	indol-acetic acid	indolacetic acid plus ascorbic acid
After soaking seeds								
Total	36.4	48.9	41.6	47.6	127.8	134.8	146.6	166.4
Free	36.1	47.2	38.2	45.6	126.6	132.1	138.8	158.2
Combined	0.3	1.7	3.4	2.0	1.2	2.7	11.7	8.2
After spraying plants								
Total	35.4	44.8	37.4	42.6	139.4	169.0	144.2	160.1
Free	34.9	42.6	32.1	39.4	137.8	164.4	136.6	158.0
Combined	0.5	2.2	5.3	3.2	1.6	4.6	7.6	2.1

A similar result was also established in the radish plants (Table 2) — the largest ascorbigen content in leaves as well as in the edible roots was found in the variant of seeds treated by indolacetic acid, next in the variant treated with a mixture of indolacetic acid and ascorbic acid; its content was lowest in the control.

The spraying of growing lettuce and radish plants was conducted with the same solutions used to soak the seeds. The treatment of lettuce was conducted on the 5th and 18th day, and that of radish on the 3rd and 14th day after sprouting. Determination of ascorbic acid content was conducted the 22nd day after sowing.

As is seen from data in Tables 1 and 2, in the same way as when the seeds are soaked, the largest vitamin C content is noted when plants are sprayed with ascorbic acid and a mixture of ascorbic and indolacetic acids. The largest ascorbigen content is found when plants are sprayed with indolacetic acid alone. In all experimental variants the ascorbigen content is considerably larger than that of the control.

It should be noted that the radish leaves contain almost 5 times as much ascorbic acid as the lettuce leaves, and their ascorbigen content is also larger; lettuce leaves have 0.4 mg %, while radish leaves contain 1.6 mg %.

LITERATURE CITED

[1] I. Matusis, Byull. Ėksptl. Biol. 20, 66 (1945).

[2] B. M. Sumtsov, Biokhimiya 12, 539 (1947).

[3] B. M. Sumtsov, Biokhimiya 13, 492 (1948).

[4] E. L. Terent'eva, Biokhimiya 18, 296 (1953).

[5] Sen-Gupta Guha, Nature 1, 974 (1938).

[6] P. Z. Holtz, Phys. Chem. 263, 187 (1940).

[7] Reedman and McHenry, Biochem J. 32, 85 (1938).

[8] M. Valenta, M. Kutacek, and F. Icha, Sbor. Ceskoslovenske Akad. zemed. ved. Rocnik 29, 193 (1956).

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NITROGEN NUTRITION OF RICE IN ENVIRONMENTS OF KZYL - ORDA REGION

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Of the total area under rice cultivation in the USSR, 22 % is in Kazakhstan. It is concentrated chiefly in the Kzyl-Orda region. When the Kzyl-Orda dam is opened the area under rice may be increased more than 2.5 times. In this connection we conducted experiments to study the biological role of nitrogenous fertilizers for creating high rice yields in the Kzyl-Orda region.

The experimental sector is situated on the soil of the "Communism" collective farm of the Syr-Darin district. This district is typical of the district's central zone in its soil-climatic conditions. The climate here is markedly continental with a yearly moisture precipitation of 100-120 mm. Rains fall for the most part during the spring-winter season. A frostless period lasts 140-180 days. The sum of active temperatures is 3200-3800°.

On the whole the climate of the Kzyl-Orda region, as to temperature conditions, dry, sunny weather and abundant light is very favorable for rice cultivation. As an unfavorable factor can be regarded only the frequent blowing of dry northeastern winds from the Betpak-Dala desert, which may cause collapse of plants and sheddings of rice grain. For rice cultivation in this region the swampy meadow soils are used, which , as Borovskii [1] points out, are characterized by an even, level appearance, a nearby level of surface waters and a heavy mechanical consistency. Such soil characteristics considerably facilitate the cultivation of rice under continual flooding.

The experiment, repeated twice, was conducted on areas of 1000 m^2 plots of land. The experimental scheme was as follows: 1) control (without fertilizer); 2) with ammonium sulfate—120 kg of active material per hectare; 3) ammonium sulfate—80 kg ; 4) ammonium nitrate—80 kg.

The subject of the experiment was a productive fast-ripening variety of rice—Dubovskii 129, suitable for the Kzyl-Orda region. Seeds were planted during the first 10 days of May. The experiment was conducted with controlled agrotechnique, with constant flooding by a layer of water up to 20 cm. Fertilizer was added by hand

TABLE 1

Yield of Rice-Shale Depending on Different Norms and Types of Nitrogenous Fertilizers (centners per hectare)

Experimental variants	Prior crop	
	corn	Ploughed over alfalfa
Control (without fertilizer)	15.0	38.4
120 kg ammonium sulfate	50.1	90.0
80 kg ammonium sulfate	38.7	—
80 kg ammonium nitrate	21.5	—

TABLE 2

Structural Changes in Rice as Influenced by Nitrogenous Fertilization

Index (per single plant)	Control (without fertilization)	Ammonium sulfate, 120 kg of N/hect.
Plant height, cm	97.2	118.2
Number of stems (bushiness)	2.8	4.0
Number of leaves	9.6	13.5
Leaf length, cm	28.4	36.6
Number of panicles	2.0	3.6
Number of grains in stem of main panicle	85.0	122.0

TABLE 3

Changes in Dry Substance Content of Rice Plants as Influenced by Nitrogenous Fertilization at Various Phases of Development (in grams, in air-dried state per plant)

Experimental variant	Investigated items	Bushiness	Tubulation	Flowering	Waxy ripeness
Control (without fertilizer)	Roots	0.11	0.17	0.22	0.09
	Stems	—	0.10	0.71	0.90
	Leaves	0.54	0.98	1.54	0.99
Ammonium sulfate 120 kg nitrogen per hectare	Roots	0.23	0.47	0.44	0.18
	Stems	—	0.46	1.54	1.66
	Leaves	1.66	2.95	4.01	3.36

TABLE 4

Changes in Chlorophyll Content in Rice Leaves as Influenced by Nitrogenous Fertilization at Various Phases of Development (in % of air-dried weight)

Experimental variant	Leaf level	Bushiness	Tubulation	Flowering	Waxy ripeness
Control (without fertilizer)	Lower	2.8	0.55	0.32	—
	Upper	1.39	—	0.94	1.05
Ammonium sulfate 120 kg nitrogen per hectare	Lower	3.76	4.87	5.12	1.84
	Upper	4.12	5.66	5.25	3.14

into the water at the 3-4-leaf phase in a single operation. In choosing the timing for fertilizing we were guided by recommendations of Erygin [2] and Artemenko [3]. The yield obtained under these conditions is shown in Table 1.

The data in Table 1 indicate that nitrogenous fertilization gives a considerable increase in rice yield. Ammonium sulfate increased the rice yield more than 2-to 3-fold compared with the control. Ammonium nitrate increased it by only 6.5 centners/hectare. The best form of nitrogenous fertilizer was ammonium sulfate. This is explained by the fact that in flooding the rice field with water anaerobic conditons are created. Under such conditions due to absence or insufficient supply of oxygen, reduction processes appear and the nitrate nitrogen, reduced to the free state, is lost to the rice plants. The loss of the nitrate form of nitrogen may be partially due to washing out. These hypotheses can be affirmed by studies of Sharapov [4], who noted the disappearance of nitrates even 5-8 days after rice field flooding.

The preference of ammonium sulfate over ammonium nitrate is also due to the fact that the first salt is physiologically acid. As indicated by Pryanishnikov [5], Maksimov [6] and others, physiologically acid salts help in weakening the alkaline reaction in alkaline soils and tend to acidify it. Such conditions are more favorable for plant growth and development.

Thus, ammonium sulfate is the best form of nitrogenous fertilizer to increase yield of rice cultivations with continuous flooding. This was confirmed over many years in studies of other authors [7-9].

Another picture is found in dry rice cultivation. Gushchin [7], Dzhulai [10] and others found a better response in rice cultivated without flooding to ammonium nitrate. Under these conditions the rice plant assimilates both ammoniacal and nitrate forms of nitrogen. Rice plants, depending on cultivation conditions, develop a unique relation to different forms of nitrogen nutrients.

TABLE 5

Changes in Content of Total Nitrogen in Rice Organs as Influenced by Nitrogen Fertilization and Phases of Development (in % of air-dried weight)

Experimental variants	Investigated items	Bushiness	Tubulation	Flowering	Waxy ripeness
Control (without fertilizer)	Roots	0.95	0.73	0.71	0.64
	Stems	—	1.04	0.50	0.42
	Leaves	1.32	1.15	0.90	0.70
Ammonium sulfate 120 kg nitrogen per hectare	Roots	1.96	1.37	1.19	0.94
	Stems	—	1.13	0.71	0.55
	Leaves	3.01	2.88	2.45	1.21

TABLE 6

Changes in Carbohydrate Content of Rice Organs as Influenced by Nitrogenous Fertilizers (in % of air-dried weight)

Development phases	Monosaccharides	Disaccharides	Maltose-like sugars	Starch	Total carbohydrates
			Leaves (control)		
Bushiness	2.2	5.8	0.6	12.6	21.2
Tubulation	2.3	4.1	1.1	11.2	18.7
Flowering	1.8	4.0	2.8	10.8	19.4
Waxy ripeness	1.7	5.5	0.4	4.1	11.7
			Stems (control)		
Tubulation	1.3	6.9	1.0	28.5	37.7
Flowering	2.2	5.8	2.9	20.9	31.8
Waxy ripeness	1 3	7.1	0.1	7.6	16.1
			Roots (control)		
Bushiness	2.3	5.4	3.0	—	10.7
Tubulation	1.1	6.6	1.8	7.8	17.3
Flowering	3.7	4.3	1.7	3.1	12.8
Waxy ripeness	0.5	4.2	0.5	2.1	7.3
			Leaves (experimental)		
Bushiness	2.3	5.7	1.9	2.1	12.0
Tubulation	3.7	5.3	0.4	5.4	14.8
Flowering	3.5	3.8	0.4	5.6	13.3
Waxy ripeness	1.8	4.4	0.2	4.3	10.7
			Stems (experimental)		
Tubulation	2.4	3.4	1.4	12.1	19.3
Flowering	3.0	3.4	1.1	10.9	18.4
Waxy ripeness	2.4	5.2	0.7	4.2	12.5
			Roots (experimental)		
Bushiness	1.0	2.0	0.7	—	3.7
Tubulation	3.0	2.9	0.4	2.3	8.6
Flowering	1.8	4.8	0.6	1.6	8.8
Waxy ripeness	0 3	2.5	0.1	3.1	6.3

In noting the considerable increase of rice yield through the use of ammonium sulfate, it is quite important to learn to what structural changes in the plant this high yield is due (Table 2).

The data in Table 2 indicate that ammonium sulfate causes an increase in all plant organs compared to the control. The bushiness of rice is especially increased, which gives a greater density in stand of plants. The nitrogenous fertilization helps increase the numbers of panicles per plant, and also increases the number of stems in the rice panicles. The large number and size of leaves is conducive to the photosynthetic process and secures a greater accumulation of dry substance by the rice plant in the vegetative process. This can be seen from data of Table 3.

Chlorophyll plays a most important part in the life of plants. It is known that up to 95 % of dry substance (carbohydrate) of the plant is synthesized by photosynthesis [6].

Determination of chlorophyll content in leaves of the experimental variants also shows a large difference (Table 4).

From Table 4 it can be seen that the chlorophyll content during all phases of rice development was higher in the experimental plants than in the control. The highest chlorophyll content is noted in phases of tubulation and rice flowering. During rice ripening the chlorophyll content is markedly decreased, which, evidently, is explained by the aging of leaves and chlorophyll destruction.

Among the elements of plant nutrients nitrogen occupies a special place. Analytical data confirmed that nitrogenous fertilizers cause essential changes in the nitrogen content of all organs in rice plants (Table 5).

Data in Table 5 indicate that fertilizers change the plant's nitrogen metabolism. The highest nitrogen content is noted in the leaves of experimental rice plants, followed by the roots and stems. In the initial phases of the plant's growth and development more nitrogen is found than in subsequent ones. This indicates a flow of nitrogenous substances into the reproductive organs [11-13].

It is known that the plant's nitrogen metabolism is closely related to the carbohydrate metabolism. The data on changes of carbohydrate content by nitrogenous fertilizers is reported in Table 6.

Data in Table 6 indicate that the total carbohydrate content is highest in rice stems, followed by leaves and roots. At the same time there are more carbohydrates in all organs of control plants compared to the experimental. This is evidently explained by the fact that in the presence of sufficient nitrogen the carbohydrates are consumed more quickly and in greater quantities in forming the plant's plastic substances. It should be noted that in the initial phases of growth and development the rice plants contain more carbohydrates as well as nitrogenous substances than in later ones.. This refers to both the experimental and control plants, which once more indicates the reuse of carbohydrates from the vegetative to reproductive organs as the plants ripen. Consequently, the nitrogenous fertilization of rice plants causes a change in the carbohydrate balance.

While conducting the study of fertilizer effect on rice yield , we simultaneously traced its effect on grain quality. As a result an increase of nitrogen content and consequently of protein was found in the rice grain. Thus, the grains of the experimental plants contained 1.6 % total nitrogen, which is equivalent to 10 % protein (as raw protein), while the control contained respectively 1.4 and 8.7 %. The starch content in rice grains was 72.5 % with nitrogen fertilizer, 67.8 % in the control.

Thus, nitrogenous fertilizers cause deep-seated physiological-biochemical changes in plants, changing their carbohydrate and nitrogen metabolism, as a result of which there is an increased yield and improved quality of this grain. This has been confirmed by experimental work over a number of years (1955-1958).

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LITERATURE CITED

[1] V. M. Borovskii, New Methods in Rice Agrotechnique [in Russian] Izd. AN KazSSR (1950).

[2] P. S. Erygin and E. F. Tishina, Transactions of the Kuban Scientific-Research Institute [in Russian] 1, (29) (Krasnodar, 1954).

[3] G. P. Artemenko, Transactions of the Kuban Scientific-Research Institute [in Russian] No. 1 (29) (Krasnodar, 1954).

[4] L. D. Sharapov, Transactions of the Scientific Conference on Results of Soil Melioration Studies of the Kazakh SSR Academy of Sciences in Downstream Rivers of Syr-Dar'i for 1946-1956 [in Russian].

[5] D. N. Pryanishnikov, Agrochemistry [in Russian] (Sel'khozgiz, 1952).

[6] N. A. Maksimov, Short Course in Plant Physiology [in Russian] (Sel'khozgiz, 1958).

[7] G. G. Gushchin, Rice [in Russian] (Sel'khozgiz, 1938).

[8] B. A. Neunylov, Doctoral Diss. Author's report (1956) [in Russian].

[9] K. S. Kirichenko, Agrotechnique of High Rice Yield [in Russian] (Sel'khozgiz, 1949).

[10] A. P. Dzhulai, Rice Cultivation without Flooding [in Russian] (Sel'khozgiz, 1953).

[11] D. A. Sabinin, Mineral Plant Nutrition [in Russian], Izd. AN SSSR (1940).

[12] E. I. Ratner, Mineral Plant Nutrition and Adsorptive Capacity of Soil [in Russian], Izd. AN SSSR (1950).

[13] E. I. Ratner, Plant Nutrition and Use of Fertilizers [in Russian] , Izd. AN SSSR (1955).

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STRUCTURE AND PHYSIOLOGICAL ACTIVITY OF SOME 2, 4, 5-TRICHLORPHENOXYACETYLAMINO ACIDS

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Many halogenated phenoxyacetic and halogenated phenoxypropionic acids are characterized by high physiological activity and are widely used as stimulators of physiological processes and as herbicides. Perhaps the widest application of the acids mentioned are those of 2,4,5-trichlorphenoxyacetic and 2,4,5-trichlorphenoxy-α-propionic. In small doses the first is used chiefly as a means of accelerating the ripening and increasing the yield of tomatoes [1,2,3], and the second to decrease the preharvest dropping of fruit from apple and pear trees [4,6]. The acids are used in large doses as herbicides [7,8].

To clarify the mode of action of the mentioned phenoxy compounds, it is of great interest to study their fate in plants [3,6,9,10]. One of the probable conversions of halogenated phenoxyalkylcarbonic acids in the plant may be their participation in protein metabolism, reaction with amino acids and derivatives of amino acids with the formation of corresponding halogenated phenoxyacylamino acids.

Recently it was shown that a 3-indolyl-acetylaspartic acid [11] can be formed in plants. In the literature there are also described some similar derivatives of 2-methyl-4-chlorphenoxyacetic acid and 2,4 dichlorphenoxypropionic acid with amino acids [12, 16]. It was of interest to synthesize and study some 2,4,5-trichlorphenoxyacetylamino acids not described in the literature. The synthesis of 2,4,5-trichlorphenoxyacetylamino acids was accomplished by reaction of a chloranhydride of 2,4,5-trichlorphenoxyacetic acid with corresponding amino acids [12].

In view of the fact that tyrosine, unlike the other amino acids used in synthesis, does not produce the one desired compound on reaction with the chloranhydride of 2,4,5-trichlorphenoxyacetic acid, we had to use tyrosine in the form of its methyl ester and synthesize the methyl ester N, O-bis-2,4,5-trichlorphenoxyacetyl-D,L-tyrosine. The compounds we synthesized and their properties are shown in Table 1.

All the synthesized compounds were tested in different concentrations on Rosovo-krasnyi sprouting radish seeds and Moskovka wheat. The seeds were soaked in the solutions for 24 hours. They were then rinsed three times in distilled water and were placed in Petri dishes on moistened filter paper. Each concentration was tested in triplicate (3 Petri dishes).

Fifty seeds were tested in each replicate. Seed germination was conducted in diffused light at 18-20°. The degree of the tested compounds' activity was judged by the length of the roots and the stem portion of sprouts. In radish sprouts the lengths of stems and the stem root, while in wheat sprouts the leaves and the total length of all rootlets of the root fibers were calculated. Measurements were made the 8th day after the start of seed soaking.

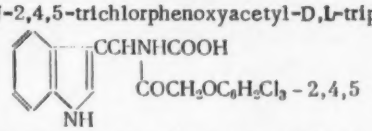
Since the methyl ester of N,O-bis-2,4,5-trichlorphenoxyacetyl-D,L-tyrosine is insoluble in water, an auxiliary substance OP-10 was added and an aqueous emulsion was prepared from the mixture. The investigated preparation and OP-10 were used in proportion of 1:1. If, for example, the preparation in the emulsion was present in a concentration of 10^{-1}, then the OP-10 concentration was the same. In comparing the physiological

TABLE 1

Newly Synthesized Compounds and Their Properties

Preparation No.	Name and formula	Melting point, °C	$[\alpha]_D^{20}$ in dioxane	% yield
1	N-2,4,5-trichlorphenoxyacetylglycine 2,4,5-$C_6H_2Cl_3OCH_2CONHCH_2COOH$	195.5—196.5	—	85
2	N-2,4,5-trichlorphenoxyacetyl-D,L-α-aminopropionic acid 2,4,5-$C_6H_2Cl_3OCH_2CONH$ $\diagdown$CHCOOH / CH_3	206	—	81
3	N-2,4,5-trichlorphenoxyacetyl-β-aminopropionic acid 2,4,5-$C_6H_2Cl_3OCH_2CONHCH_2CH_2COOH$	172	—	82
4	N-2,4,5-trichlorphenoxyacetyl-D,L-valine 2,4,5-$C_6H_2Cl_3OCH_2CONH$ $\diagdown$CHCOOH / $(CH_3)_2CH$	194.5—195.5	—	90
5	N-2,4,5-trichlorphenoxyacetyl-L-leucine 2,4,5-$C_6H_2Cl_3OCH_2CONH$ $\diagdown$CHCOOH | $(CH_3)_2CHCH_2$	154—155	—5.8°	92
6	N-2,4,5-trichlorphenoxyacetyl-D,L-phenylalanine 2,4,5-$C_6H_2Cl_3OCH_2CONH$ $\diagdown$CHCOOH / $C_6H_5CH_2$	200	—	86,2
7	N-2,4,5-trichlorphenoxyacetyl-D,L-serine 2,4,5-$C_6H_2Cl_3OCH_2CONH$ $\diagdown$CHCOOH / $HOCH_2$	157—158	—	69
8	N,S-bis-2,4,5-trichlorphenoxyacetyl-D,L-cysteine 2,4,5-$C_6H_2Cl_3OCH_2CONH_2$ $\diagdown$CHCOOH / 2,4,5-$C_6H_2Cl_3OCH_2COSCH_2$	164	—	96.5
9	N,N'-bis-2,4,5-trichlorphenoxyacetyl-L-cystine SCH_2—CHNHOCCH$_2$OC$_6$H$_2$Cl$_3$—2,4,5 | | COOH SCH_2—CHNHOCCH$_2$OC$_6$H$_2$Cl$_3$—2,4,5 | COOH	141	—42.9°	76.5
10	$N^{\alpha}N^{\epsilon}$-bis-2,4,5-trichlorphenoxyacetyl-L-lysine 2,4,5-$C_6H_2Cl_3OCH_2CONHCH_2CH_2CH_2CH_2CHCOOH$ | 2,4,5-$C_6H_2Cl_3OCH_2CONH$	159—160	—11.2°	91.7
11	N-2,4,5-trichlorphenoxyacetyl-D,L-methionine 2,4,5-$C_6H_2Cl_3OCH_2CONH$ $\diagdown$CHCOOH / $CH_3SCH_2CH_2$	123—124	—	—

TABLE 1 (continued)

Preparation No.	Name and formula	Melting point, °C	$[\alpha]_D^{20}$ in dioxane	% yield
12	N-2,4,5-trichlorphenoxyacetyl-D,L-aspartic acid $2,4,5\text{-}C_6H_2Cl_3OCH_2CONH$ $\diagdown CHCOOH$ $HOOCCH_2\diagup$	167—169	—	69.2
13	N-2,4,5-trichlorphenoxyacetyl-L-glutamic acid $2,4,5\text{-}C_6H_2Cl_3OCH_2CONH$ $\diagdown CHCOOH$ $HOOCCH_2CH_2\diagup$	123—124	—10.5°	76
14	N-2,4,5-trichlorphenoxyacetyl-D-glutamic acid $2,4,5\text{-}C_6H_2Cl_3OCH_2CONH$ $\diagdown CHCOOH$ $HOOCCH_2CH_2\diagup$	123—124	+10.6°	66.7
15	N-2,4,5-trichlorphenoxyacetyl-D,L-glutamic acid $2,4,5\text{-}C_6H_2Cl_3OCH_2CONH$ $CHCOOH$ $HOOCCH_2CH_2$	127.5	—	45.4
16	N-2,4,5-trichlorphenoxyacetyl-D,L-triptophane  $CCHNHCOOH$ $COCH_2OC_6H_2Cl_3\text{-}2,4,5$ NH	90 With decomposition	—	94.1
17	Methyl ester N,O-bis-2,4,5-trichlorphenoxyacetyl-D,L-tyrosine $2,4,5\text{-}C_6H_2Cl_3OCH_2COOC_6H_4-CH_2$ $\diagdown CH$ $2,4,5\text{-}C_6H_2Cl_3OCH_2CONH\diagup$ $\diagup$ $COOCH_3$	120—123	—	—

activity of synthesized compounds and 2,4,5-T all the substances were taken in the form of diethanolammonium salts. Results of the experiments are given in Tables 2,3 and 4. All concentrations of substances are given in percentages (gm per 100 ml water), calculated as 2,4,5-trichlorphenoxyacetic acid.

As seen from Table 2, many 2,4,5-trichlorphenoxyacetylamino acids in concentrations of 10^{-3} to 10^{-1}, calculated as 2,4,5-T, inhibit radish growth. Herbicidal activity of compounds with L-leucine, D,L-phenylalanine, D,L- cysteine,L-cystine, L-lysine, D,L-methionine, D-glutamic acid, D,L-tryptophan, D,L-glutamic and D,L-aspartic acids exceeds activity of 2,4,5-T. In concentration of 10^{-4} %,compounds with D,L-valine, D,L--serine and D,L-aspartic acid stimulate radish growth, while the other acylamino acids inhibit growth of sprouts.

In testing 2,4,5-trichlorphenoxyacetylamino acids on wheat sprouts (Table 3) for herbicidal activity in concentrations of 10^{-1} the compounds with D,L-serine, D,L-cysteine, L-cystine, D,L-aspartic acid and D-glutamic acid exceed the activity of 2,4,5-T. The methyl ester N, O-bis-2,4,5-trichlorphenoxyacetyl-D,L-tyrosine proved to be more active than 2,4,5-T in tests on both radish and wheat sprouts.

Wood and Fontaine [13] and Krewson and others [14, 15] expressed the opinion that the herbicidal activity of halogenated phenoxyacetylamino acids depends on the presence of hybrolytic enzymes in plants capable of splitting the amide bond of L- and D,L-amino acid derivatives with the formation of halogenated phenoxyacetic acids and incapable of hydrolyzing the amide bond of D-amino acid derivatives. However, the high herbicidal

TABLE 2

Physiological Activity of 2,4,5-Trichlorphenoxyacetylamino Acids (test-item: radish sprouts; activity calculated by average length of root and stem, mm)

Preparation No.	Preparation	M	Concentration of compared preparations							
			10^{-1}		10^{-2}		10^{-3}		10^{-4}	
			stem	root	stem	root	stem	root	stem	root
1	T-glycine	312.543	—2.5	2.0	5.4	—	5.7	17.3	11.3	26.7
2	T-D,L-α-aminopropionic	326.569	—2.5	2.5	4.5	12.7	10.2	27.8	6.7	30.6
3	T-β-aminopropionic	326.569	—2.2	1.5	4.9	4.2	6.2	9.7	8.9	17.9
4	T-dl-valine	338.641	—2.5	2.9	6.1	17.2	6.0	16.7	13.6	41.4
5	T-D,L-leucin	368.647	0	0	4.5	12.2	10.7	25.4	10.0	22.8
6	T-D,L-phenylamine	402.661	1.3	0.6	4.2	10.0	7.3	19.1	10.4	33.7
7	T-D,L-serine	342.569	1 8	1.1	4.8	21.0	5.9	23.4	13.2	47.1
8	T-D,L-cysteine	596.140	0 6	0.4	4.4	9.7	7.2	16.9	6.2	16.6
9	T-L-cystine	715.242	0	0	4.4	10.0	6.4	15.2	7.1	11.1
10	T-L-lysine	621.138	0.3	0.08	4.5	9.0	4.8	14.3	8.8	17.6
11	T-D,L-methionin	886.681	0	0	4.2	9.6	7.1	22.6	8.8	19.2
12	T-D,L-aspartic acid	370.579	1.4	1.0	4.8	18.6	12.8	51.8	12.5	53.5
13	T-L-glutamic acid	384.605	—2.9	2.0	4.7	12.9	10.4	23.2	—	—
14	T-D-glutamic acid	384.605	—	—	1 1	0.7	3.4	7.7	6.6	20.6
15	T-D,L-glutamic acid	384.605	0.07	0.07	3.9	10.3	6.1	14.1	7.7	18.8
16	T-D,L-tryptophan	441.697	1.4	1.0	4.5	13.0	6.8	13.1	8.3	24.8
17	2,4,5-trichlorphenoxyacetic acid	255.5	1.6	1.3	6.7	24.0	17.1	50.9	14.8	47.2
18	Water	—	10.0	33.1	—	—	—	—	—	—

TABLE 3

Physiological Activity of 2,4,5-Trichlorphenoxyacetylamino Acids (test-item: wheat sprouts; activity calculated by the average length of roots and sprout, mm)

Preparation No.	Preparation	M	Concentration of compared preparations							
			10^{-1}		10^{-2}		10^{-3}		10^{-4}	
			sprout	roots	sprouts	roots	sprouts	roots	sprouts	roots
1	T-glycine	312.543	52.9	−21.9	—79.5	125	81.0	183.5	92.0	220
2	T-D,L-α-aminopropionic	326.569	42.0	3.8	—71.5	90.5	−114	203	91.3	167
3	T-β-aminopropionic	326.569	51.0	—18.7	−69.0	78.5	—87.3	228	85.7	197
4	T-D,L-valine	338.641	50.9	—30.3	—81.0	123.1	—87.9	183.0	92	221
5	T-L-leucine	368.647	47.7	−14.6	—63.5	89.2	+89.8	139	86.4	199.5
6	T-D,L-phenylalanine	402.661	66.1	−47.2	—84.2	- 156	85	197	- 93.0	224
7	T-D,L-serine	342.569	12.0	2.36	+51.9	—143	84.5	175	84.9	161
8	T-D,L-cysteine	596.140	19.1	2.45	56.0	55.5	87.3	196	88.9	199
9	T-L-cystine	715.242	17.0	2.76	48.5	44.6	86.4	151	83.7	174
10	T-L-lysine	621.138	54.3	—25.9	—73.6	−156.8	—92.0	199	89.1	208
11	T-D,L-methionine	386.681	46.5	—13.4	−74.3	- 79.0	−93.5	238	—94.0	198
12	T-D,L-aspartic acid	370.579	23.0	2.5	+49.5	66.7	83.0	214.0	89.6	230.5
13	T-L-glutamic acid	384.605	49.3	—4.5	—58.5	—87.2	—90.5	200	83.9	188.5
14	T-D-glutamic acid	384.605	0.73	0.7	+27.8	5.4	68.5	221.0	82.4	183.0
15	T-D,L-glutamic acid	384.605	35.0	3.25	—67.0	- 87.9	- 89.1	204,0	92	208.0
16	T-D,L-tryptophane	441.697	71.5	−122.9	—86.3	— 180	—93.6	185	89.5	172.5
17	T	225.5	33.3	4.0	63.0	64.1	83.0	153	89.6	222
18	Water	—	97.8	232	—	—	—	—	—	—

TABLE 4

Physiological Activity of 2,4,5-T and Methyl Ester N,O-bis-2.4,5-Trichlorphenoxyacetyl--D,L-Tyrosine (activity is calculated by increase in length of root and stem portions of sprouts in mm, cultivated at 27° for 10 days; test-items: radish and wheat sprouts)

Names of substances	Concentration of compared preparations							
	10^{-1}		10^{-2}		10^{-3}		10^{-4}	
	stem	root	stem	root	stem	root	stem	root
Methyl ester N,O-bis-2,4,5-trichlorphenoxyacetyl-D,L--tyrosine + + OP-10	4.7	6.4	5.5	11.5	7.3	30.2	19.2	52.8
2,4,5-T	4.8	2.6	11.0	36.2	13.9	58.0	19.5	91.7
OP-10	16.4	81.7	30.6	101.1	18.1	91.5	26.4	82.0
Water	19.0	102.2	—	—	—	—	—	—
Methyl ester N,O-bis-2,4,5-trichlorphenoxyacetyl-D,L--tyrosine + OP-10	0	0	92.1	206.5	148.3	370.0	169.1	460.1
2,4,5-T	33.3	42.4	112.0	192.9	161.0	452.0	176.0	469.0
OP-10	177.9	449.0	159.6	363.0	169.0	349.5	165.0	447.1
Water	160.1	426.0	—	—	—	—	—	—

activity of 2,4,5-trichlorphenoxyacetyl-D-glutamic acid by comparison with L-derivatives, and the higher activity of 2,4,5-trichlorphenoxyacetic acid compounds with some amino acids of the L- and D,L-groups by comparison with 2,4,5-T, contradict this point of view.

Our results are in line with the theory [15] that the physiological activity of compounds depends on the molecular structure as a whole and capacity for mobility of the compound in the plant.

As seen from Tables 2 and 3, a number of 2,4,5-trichlorphenoxyacetylamino acids in concentrations of 10^{-1} and 10^{-2} inhibit the growth of roots and stem portions of radish and wheat, while in lower concentration they stimulate growth of wheat sprouts, and inhibit growth of radish sprouts and the selectivity of their action is less than in the case of 2,4,5-trichlorphenoxyacetic acid. The 2,4,5-trichlorphenoxyacetyl-glutamic and, aspartic acids inhibit the growth of stem and roots especially strongly; in their activity they considerably surpass the sodium salt of 2,4,5-trichlorphenoxy acetate, D- and L-2,4,5-trichlorphenoxyacetylglutamic acids differ little in their activity on radish roots and stems, but the D-acid inhibits the growth of wheat roots considerably more than the L-acid and racemate.

The indicated compounds act on plants similarly to the earlier described 2,4-dichlorphenoxyacetylamino acids [12].

The conducted experiments show that among the compounds of the group studied there are substances with stronger herbicidal activity than 2,4,5-T.

LITERATURE CITED

[1] Yu. V. Rakitin, Use of Growth Substances for Plants (1947) [in Russian].

[2] Yu. V. Rakitin and A. V. Krylov, Use of Growth Stimulants to Raise Tomato Productivity [in Russian] (Izd. AN SSSR, 1950).

[3] Yu. V. Rakitin, Uspekhi Sovremennoi Biol. 36 (6), 289 (1953).

[4] L. I. Edgerton and M. B. Hoffman, Proc. Amer. Soc. Hortic. Sci. 57, 120 (1951).

[5] T. W. Southwick, I. E. Demoranville, and L. F. Anderson, Proc. Amer. Soc. Hortic. Sci. 61, 155 (1953).

[6] Yu. V. Rakitin, Control of Life Activity of Plants [in Russian] (Izd. "Znanie", 1956).

[7] C. L. Hamner and H. B. Tukey, Science 100, 154 (1954).

[8] N. N. Mel'nikov, Yu. A. Baskakov, and K. S. Bokarev, Chemistry of Growth Stimulants and Herbicides [in Russian] (Goskhimizdat, 1954).

[9] Vestnik Akad. Nauk SSSR, Razdel "Pamyatnye Daty", Timiryaz, Chtenie, 17, 117 (1956).

[10] Yu. V. Rakitin, Use of Stimulants and Herbicides for Plants [in Russian] (Izd. "Znanie", Moscow, 1957).

[11] W. A. Andreae and N. E. Good, Plant Physiol. 30, 4, 380 (1955).

[12] K. S. Bokarev and N. M. Mel'nikov, Zhur. Obshchei Khim. 25, 2493 (1955).

[13] J. W. Wood and T. D. Fontaine, Organ Chem. 17, 891 (1952).

[14] C. F. Krewson, C. H. H. Neufeld, T. F. Drake, T. D. Fontaine, J. W. Mitchell and W. H. Preston, Weeds 3, 28 (1954).

[15] C. F. Krewson, T. F. Drake, C. H. H. Neufeld, T. D. Fontaine, J. W. Mitchell, and W. H. Preston, J. Agric. and Food Chem. 4, 140 (1956).

[16] C. F. Krewson, T. F. Drake, J. W. Mitchell and W. H. Preston, J. Agric. and Food Chem. 4, 690 (1956).

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TRANSPIRATION AND CELL SAP CONCENTRATION OF CERTAIN FODDER PLANTS UNDER DESERT CONDITIONS IN UZBEKISTAN

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In the deserts of Uzbekistan, particularly in the ephemeral Artemesia desert, the average yearly precipitation is 140-160 mm with fluctuations from 81 to 250 mm. The average yearly temperature is 14-16°. In June and July the temperature in the shade reaches 40-46° and higher. The average relative humidity during the year is 30 %, and at the end of spring and summer it is 15 %.

In association with these xerothermic conditions a peculiar plant cover with an extremely low fodder content has been formed. For the pasturage of sheep, which are maintained the year round on green fodder, it is necessary to create reserve fodder in the winter.

The method of growing certain fodder plants in the desert zone of Uzbekistan has been developed by the Division of Pasture Development of VNIIK. Even in dry years, safflower gives up to 16 cwt./hectare, corn up to 10 cwt./hectare, and sunflower up to 30 cwt./hectare of silage. These yields under desert conditions exceed the yield of natural ephemeral Artemesia pastures by 10-fold or more, and on the basis of fodder units, by 15-fold or more.

In developing the technique of growing fodder crops under desert conditions new to them, we also studied the water regime of these plants. Soil moisture dynamics, transpiration intensity, and cell sap concentration were examined. Observations were made in 1957 on the "Karnab" state farm of the Samarkand region of the UzbSSR. The soil to be sown was plowed in December to a depth of 20-22 cm. Safflower and sunflower were sown at the end of February and corn at the end of March.

Soil moisture was studied by the usual method. Transpiration was determined by the weight method of Ivanov [1]. Leaves were removed without paraffin[Radionov 2, 3]. Measurements were made at 7 a.m., 11 a.m., 2 p.m., and 6 p.m.. The isolated leaves were weighed on torsion balances with a sensitivity of 0.5 mg, with an exposure time of 3 minutes. Measurements were replicated four or five times. Cell sap concentration was determined with a refractometer [4]. Leaves of the upper nodes (usually the fourth node from the growing point) were cut off and ground. A drop of sap extracted from the ground-up leaves was placed on a refractometer prism and the refractive index measured. Measurements were repeated four to six times.

From the data presented in Table 1 it can be seen that the highest transpiration rate in safflower, sunflower and corn occurred at midday (11 a.m. and 2 p.m.), and the lowest in the morning (7 a.m.) and evening (7 p.m.) hours.

At the beginning of the plant's growth, the transpiration rate fluctuates within quite wide limits in response to abruptly changing climatic factors. For example, on April 29 the transpiration rate in safflower at 7 a.m. was 0.529 g per hr per g dry weight, at 2 p.m.—1.718 g; correspondingly, in sunflower it was 0.436 and 2.140 g; in corn — 0.720 and 2.150 g. The expenditure of water in these plants is four to five times greater during the midday hours than in the morning and evening hours.

In all crops, with the exception of safflower, the highest transpiration rate was observed on the 29th of April and the 8th of May.

TABLE 1

Transpiration Rate in Certain Fodder Crops during the Daylight Hours on Various Dates (in g per hr per g dry wt.)

Date of measurements	Hr of measurement	Crop: safflower	Crop: sunflower	Crop: corn	Date of measurements	Hr of measurement	Crop: safflower	Crop: sunflower	Crop: corn
29.IV	7	0.529	0.436	0.720	28.V	7	0.578	0.310	0.498
	14	1.718	2.140	2.150		11	0.960	0.480	0.590
	19	0.376	0.504	1.716		14	0.612	0.650	0.294
	Average	0.874	1.026	1.198		19	0.238	0.604	0.384
8.V	7	0.550	2.210	1.672		Average	0.547	0.511	0.441
	11	0.3 6	2.680	3.320	6.VI	7	0.762	0.584	0.556
	14	0.652	1.9'0	3 330		11	0.564	0.580	0.566
	19	0.262	0.842	1.454		14	0.538	0.628	0.992
	Average	0.492	1.668	2.444		19	0.502	0.464	0.372
16.V	7	0.478	0.772	1.164		Average	0.591	0.564	0.621
	11	0.603	0.774	1.190					
	14	0.950	0.776	1.976					
	19	0.208	0.298	1.332					
	Average	0.559	0.645	1.413					

TABLE 2

Cell Sap Concentration (in % of dry weight) in Certain Fodder Crops

Crop	Date of measurement: May 5	May 27	June 6	June 21
Safflower	7.1	12.9	21.5	—
Sunflower	5.1	8.7	12.3	11.9
Corn	6.3	8.9	10.6	10.6

Conditions prevailing on these days were favorable to high transpirations. The soil moisture content was high. The moderate relative humidity and temperature of the air also favored high transpiration. On May 28 relatively low rates of transpiration were observed, but as a whole they were still fairly high. On June 6 minimal rates were observed in all crops.

Under the conditions prevailing in the ephemeral Artemesia desert at Karnab , the pattern of transpiration in safflower, sunflower and corn consists fundamentally of a maximum in the morning and evening hours. The pattern over the growth period shows a maximum at the end of April and in the first half of May and a minimum at the end of May and the beginning of June .

A comparison of transpiration indices (averages over a day) shows quite large differences among safflower, sunflower and corn. Corn transpires most intensively, then sunflower, and finally safflower, which utilizes H_2O most economically. Moreover, while corn and sunflower react strongly to changes in climatic factors both in the course of a day and during the growing period, such a behavior was not observed in safflower; both in the course of a day and of a growing period it has the highest number of values within 0.874–0.547 g per hour per g dry weight.

The osmotic pressure of cell sap is of great physiological significance in drought conditions. High osmotic pressures, which facilitate water uptake from the relatively drier soil and hinder its loss by evaporation, increase the over-all resistance of the plant to unfavorable external conditions [5, 6]. Cell sap concentrations of the three crops during the growing period are given in Table 2 .

If the cell sap concentration indices of the various crops are compared, it can be seen that corn and sunflower have the lowest values, and that safflower has significantly high values.

The properties of safflower described above (low transpiration rate and high cell sap concentration) afford a more complete and economical utilization of soil water, which also guarantees a high fodder and satisfactory seed yield in the desert zone of Uzbekistan.

LITERATURE CITED

[1] L. A. Ivanov, A. A. Silina, Yu. L. Tsel'niker, Bot. Zhur. 35, 171 (1950).

[2] M. S. Radionov, Bot. Zhur. 40, 97 (1955).

[3] M. S. Radionov, Fiziol. Rastenii 4, 107 (1956).

[4] A. I. Ermakov, V. V. Arasimovich, et al., Methods of Biochemical Investigation in Plants [in Russian] (Sel'khozgiz, 1952).

[5] B. A. Keller, Russkoe Bot. Obshchestva 5 15 (1920).

[6] S. I. Kokina, Collected Papers, Problems in Utilization of Desert for Plant Cultivation [in Russian] 1, (Izd. VASKhNIL, 1935).

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APPLICATION OF COMPLETED WORKS IN THE NATIONAL ECONOMY

WATER CONDITIONS IN A RICE FIELD DURING GERMINATION OF THE RICE SEEDS

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In a study of external conditions prevailing during rice germination, it was established that early germination begins without access of oxygen to the embryo. Under such conditions an excess of growth materials is accumulated in the embryo which inhibits the growth of the root and stimulates bud growth. In this time germination of the coleoptile proceeds only as cell elongation: Under anaerobic conditions new cells are not formed because of the deficient energy supply. Such is the mechanism of germination in early stages: It is characteristic of both submerged and moist soil. This property of germinating rice seeds may be utilized in the development of a soil water regime during the initial growing stages.

Since rice seeds do not require oxygen at the beginning of germination, it is possible to submerge them during inhibition and early germination states: Such conditions do not yield adverse effects during germination proper. The duration of submergence is half the time from sowing to the appearance of shoots. This means that the field plots may be kept submerged without injury to germinating seeds.

After the early germination stages, the embryo requires oxygen, access to which is provided by removal of water from the plots. Under these conditions the radical emerges and penetrates into the soil and the first green leaf appears; the plant thus becomes autotrophic. These shoots are then inundated in order to inhibit the growth of weeds. This water regime during the germination period is shown in Fig. 1.

Such a water regime takes into consideration the biological properties of weeds in such a way that the weeds are eliminated. If attention is directed to the ability of the seeds of these plants to germinate under conditions of limited oxygen supply, the following pattern emerges (Table 1).

Table 1 shows that seeds of rice and weed seeds germinate differently in water : Weed seeds germinate more poorly since they require more oxygen.

These data are somewhat analogous to what occurs on the surface of a submerged plot (unboiled water) and in submerged soil (boiled water). From this it is deduced that those weeds seeds which have been inundated but not planted in the soil give a considerable number of shoots. When they are embedded in submerged soil, however, they are unable to germinate as successfully as rice. Similarly, removal of water during early germination stages

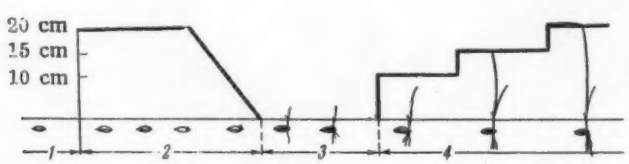

Fig. 1. Soil water regime during germination of rice seeds (schematic). 1) Sowing; 2) first flooding; 3) removal of water during growth of roots and leaves; 4) second flooding.

TABLE 1

The Ability of Seeds of Rice and of Weed Seeds to Germinate in Water with Varying Levels of Oxygen

Plants	Ungerminated seeds, %	No. of Seeds with a coleoptile length, cm			germinated seeds, %
		2.0	1.5	1.0	
Unboiled water (oxygen present)					
Weeds	67	8	20	5	33
VROS 3716	2	26	14	58	98
Krasnodar 424	8	13	9	70	92
Dubov 129	8	12	6	74	92
Boiled water (oxygen absent)					
Weeds	94	1	3	2	6
VROS 3716	3	23	17	57	97
Krasnodar 424	7	14	10	69	93
Dubov 129	6	10	8	76	94

TABLE 2

The Effect of Soil Water Regime during Germination and Shoot Growth on the Weed Level of a Rice Field (number of weeds per m^2)

Water regime during germination and shoot growth	Shoots		Beginning of tillering
	two leaves	three leaves	
Flooding of shoots at the three-leaf stage	284	362	394
Flooding with the removal of water during emergence of roots and leaves from the seed	36	24	16
Continuous submergence	31	20	14

TABLE 3

Effect of Water Regime on the Density of the Rice Stand (number of plants per m^2)

Age of rice	Soil moist during germination and shoot growth	Water removed during emergence of roots and leaves from the seed	Continuous submergence
Krasnodar 424			
Shoots—two leaves	354	345	289
Shoots—three leaves	311	320	270
Tillering (beginning)	162	290	205
Full maturity	154	285	200
Dubov 129			
Shoots—two leaves	341	388	194
Shoots—three leaves	289	289	167
Tillering (beginning)	113	230	160
Full maturity	113	227	152

is more favorable to rise, since it has already passed through initial germination stages while weed seeds have merely swelled; therefore rice seedlings penetrate the soil surface more rapidly With such a water regime, and also under conditions of uninterrupted submergence, only weed seeds not confined in the soil can germinate.

The effectiveness of this water regime in the early growth of rice was tested on plots heavily infested with weeds, principally barnyard millet, in the Kalinin collective farm or the Krasnoarmiya district.

Shoots were obtained as follows: Dry seeds were sown in rows at a depth of 1-2 cm; the plot was then flooded to a depth of 20-25 cm and allowed to remain flooded for seven days until most of the seeds had passed through initial stages of germination. The water was partially

TABLE 4

Rice Yield under Various Soil Water Regimes Maintained during Germination and Shoot Growth (in cwt./hectare)

Variety	Soil moist during germination and shoot growth	Water removed during emergence of roots and leaves from the seed	Continuous submergence
Krasnodar 424	10.5	38.5	28.0
Dubov 129	14.2	43.0	34.0

adsorbed into the soil and partially drawn off. Shoots with the first green leaf appeared on the fourth day. After the appearance of such shoots, the plot was flooded a second time. Control plots were subjected to two water regimes; constant flooding and flooding at the time of appearance of shoots with three leaves. The results were evaluated on the basis of the number of rice shoots and of weed shoots per m^2. Counts were made twice during the shoot stage and subsequently during tillering and before the harvest. The size of the yield was determined according to the hopper weight and granary weight after harvest with a combine. The experiment was replicated twice. Usually 7 million seeds per hectare were sown; the date of sowing and flooding—May 23, the varieties sown—Krasnodar 424 and Dubov 129. Participating in the experiment as technicians were Pavel Markovich Korotkii and Afanasii Afanas'evich Alesin. The head agronomist of the collective farm, Ivan Semenovich Kor'nedub, was of great help in the performance of the experiment.

The plots most heavily infested with weeds proved to be those which were flooded when shoots with three leaves had appeared. This is seen in Table 2, in which the result of a count of the number of weeds occurring in a sowing of Krasnodar 424 rice is presented. The level of weed infestation on plots sown with Dubov 129 rice was approximately the same.

As Table 2 shows, the difference in weediness between plots is very large. It should be pointed out that with continuous submergence and with removal of water during germination the weeds were represented only by rice millet and Szhat millet* , i.e., by forms similar to rice in their resistance to flooding .

The density of the rice stand is presented in Table 3 .

Table 3 shows that when the soil was kept moist but not flooded during germination and when it was continuously submerged, the thinning out patterns were widely different. Thus, if numbers of plants in the two-leaf stage are compared it is seen that there are more shoots on moist soil than on flooded soil. Later, because of the exceptional weediness on moist soil, the number of rice shoots declined. On constantly flooded soil, the maximum number of seeds died during germination because of the oxygen deficiency. At the beginning of tillering and before harvest the stand was most dense when water was removed in the period of root and leaf emergence from the seed.

Stand density was correlated with yield as Table 4 shows.

Table 4 indicates that the water regime in which a first flooding is followed by removal of water during root growth is by far the most effective. The physiological peculiarities of rice germination are taken into account in this regime. It is of interest in cultivation and is recommended for use in the collective farms and state farms of the Krasnodar area.

SUMMARY

A study of internal conditions for germination of rice seeds permits one to recommend the following water regime at the beginning of vegetation of the rice. 1) Flooding of the field immediately after sowing until swelling and early germination take place. 2) Removal of water from the field plots after germination begins, the plants remaining unsubmerged until formation of the first green leaf for promotion of growth of the roots. 3) A second flooding at the time of the leaf formation, the field remaining submerged until disappearance of weeds.

* Transliteration of Russian—Publisher's note.

METHODS

BIOLOGICAL TEST FOR GIBBERELLINS

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Although gibberellins are similar to auxins in their ability to stimulate cell elongation, a comparison of their physiological activity with that of an auxin such as 3-indoleacetic acid (IAA) shows great differences. The most important differences, which are related mainly to growth phenomena, are summarized in Table 1; they have been assembled from published data.

The comparisons of Table 1 definitely indicate that the mechanism of action of these stimulators is different and that gibberellin activity must be distinguished from auxin activity. It follows that a biological test for gibberellins should be specific for them in the presence of IAA. Materials with gibberellin activity are found in a number of higher plants [1,2,15,16,17,18,19,20] and in certain microorganisms [1, 2, 21], but only one substance, isolated from unripe beans, has been identified as gibberellin A_1 [22, 23]; thus, the possibility of detecting these substances by chemical means is still limited. Even in the case of substances such as gibberellic acid (GA), whose structure is largely known, the use of a biological assay has not declined, since this test has a very high sensitivity which colorimetric and fluorometric methods cannot match [24].

Different workers have used different biological assays to detect materials with gibberellin activity. The effect of gibberellins on dwarf forms of pea and corn, and also on the rosette stage of biennial long-day plants, was utilized as a very specific test (see Table 1). Even though IAA is completely inactive in these tests, they are poorly suited for constant laboratory determinations because of their cumbersome character and the time required to perform them. Therefore tests which were more rapid and easier to perform were proposed.

Portions of leaves from wheat seedlings were employed in a test devised by Radley [14, 16]; in Nitsch's test, [18] leaf segments from oat seedlings were used; cucumber shoots were employed in the test of Galun [26]. A common disadvantage in these tests is the very weak reaction exhibited; even at optimal concentrations of GA the difference from the control is not over 3-4 mm. Moreover, Radley's test is not specific with respect to IAA, and Galun's test is difficult to utilize in the laboratory because a large number of cucumber plants are required to provide a sufficient number of straight shoots. In the recently described test of Aytoun, Dunn and Sellor [27], the material employed consists of rice seedlings whose roots are immersed in the experimental solution. Each vessel containing six seedlings requires 8 ml of solution. Although growth in comparison with the control is sufficiently high (up to 20 mm), the necessity of using large amounts of experimental solution, of carefully maintaining the temperature at 29-30°, and of providing constant illumination during the experiment, and also the variability of the results obtained, make this a very unsatisfactory test.

In seeking to develop a biological test for gibberellins which is specific and easy to perform in the laboratory, we tried a number of materials: hypocotyls and epicotyls of pea seedlings, cucumber and sunflower cotyledons, and leaves of wheat, barley and corn seedlings. In the course of these preliminary experiments we discovered an extremely interesting fact deserving of a separate, more detailed communication. If sections of leaves (together with the coleoptile) are removed from seven day-old etiolated corn seedlings at a point above the growing point, which is in turn above the mesocotyl, the reaction to GA, even at optimal concentrations, is very weak (3-4 mm); it becomes weaker the farther from the growing point the sections were taken. This is in accord with the results of Radley [16], obtained with leaf sections from wheat.

If, however, a small portion of the mesocotyl containing the point of growth is removed with the leaf section, the reaction with GA becomes very pronounced, and differences with the control reach levels 8-10 times higher; the presence of IAA is manifested only as a weak inhibition, but not a stimulation, of growth.

TABLE 1

The Chief Differences in the Effect of Heteroauxin and Gibberellins on Plants

No.	Heteroauxin	Gibberellins	Literature citations
I	The curve showing the relationship between concentration and growth has a clear optimum. High concentrations are toxic.	The curve showing the relationship between concentration and growth has as a rule a flat plateau region. High concentrations are not toxic.	[1, 2, 3]
II	Does not stimulate stem growth of intact plants in the light; specifically, does not induce shoot formation in rosettes of annual and biennial long-day plants.	Strongly stimulates stem growth of intact plants in the light; specifically, induces shoot formation in rosettes of annual and biennial long-day plants.	[1, 2, 4, 5, 20, 21]
III	Has no effect on genetic dwarf forms (peas, corn, etc.)	Converts dwarf forms to forms of the size of the original phenotypes.	[1, 2, 6, 7, 8, 9, 16]
IV	When applied to decapitated stem tips, inhibits the development of lateral buds.	When applied to decapitated stem tips, stimulates the growth of lateral buds.	[1, 2, 10, 13]
V	Gives a positive reaction in the coleoptile test and in Went's pea test.	Gives no reaction with these tests.	[1, 2]
VI	Polarity of transport clearly evident.	No polarity of transport.	[1, 2, 11, 12]
VII	Stimulates root formation.	Does not stimulate root formation and even inhibits it.	[1, 2, 10, 12, 13]
VIII	Causes epinasty in leaves.	Does not cause epinasty in leaves.	[1, 2]
IX	Does not cause chlorosis.	Causes chlorosis.	[1, 2, 4]
X	Has no effect on dormancy in potato tubers.	Induces sprouting in potatoes.	[1, 2, 10]

Especially marked contrasts are obtained if sections 3 cm long, as shown in Fig. 1, are taken. Thus, the previously described [2,28] and as yet unexplained effect of the growing point on the stem's reaction to GA is also manifested with respect to leaves. This effect provides the basis of the development of a new biological test for gibberellins.

DESCRIPTION OF METHODS

In the development of our test we used a preparation of gibberellic acid (GA) from the English firm Imperial Chemical Industries, Ltd.

In view of the fact that one deals with very large dilutions of gibberellins and auxins, it is more convenient to designate concentrations by the method employed in physical chemistry for designating concentrations of hydrogen ions, i.e., by the positive logarithm of the concentration—pC. A comparison of this method with others used in the literature is given in Table 2.

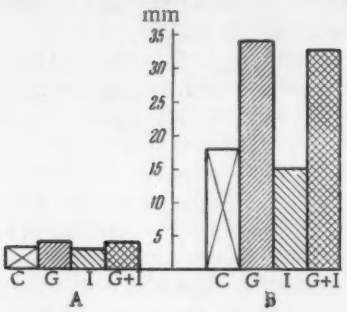

Fig. 1. The effect of the growing point on the growth of sections from the first leaf of Voronezhskaya 76 corn seedlings in solutions of GA, IAA, and GA + IAA. Length of sections 30 mm, height of bars proportional to the increase over the original length. A) Sections without a growing point, taken at 5 mm above it; B) sections with a growing point, leaves sectioned at 25 mm above it, and mesocotyl at 5 mm below it; C) control (water); G) gibberellic acid; I) indolacetic acid; G + I) mixture of GA and IAA. Concentration of both acids in all solutions: pC 6.0.

TABLE 2

A Comparison of Various Methods of Designating Concentrations of Growth Stimulators with the Method Used in This Paper

Wt /vol				
pC	10^{-a}	%	ppm*	μg /ml
4.0	10^{-4}	0.01	100.0	100.0
5.0	10^{-5}	0.001	10.0	10.0
6.0	10^{-6}	0.0001	1.0	1.0
6.3	$5 \cdot 10^{-7}$	0.00005	0.5	0.5
6.7	$2 \cdot 10^{-7}$	0.00002	0.2	0.2
7.0	10^{-7}	0.00001	0.1	0.1

* 1 ppm (parts per million) = one millionth.

Fig. 2. Apparatus for experiments. A) Flat for growing seedlings; B) glasses containing sections; left) control (water), right) GA solution, pC 6.0.

In the quantitative estimation of the activity of chemically unidentified gibberellin-like materials (cf. below), we use the symbol pA, which is equal to the pC of a solution of gibberellic acid giving the same growth effect as the experimental solution. This method, used by us for auxins in 1947 [25], makes it possible to talk in terms of concentrations where the experimental material is unidentified.

It is convenient to use a GA stock solution of pC 4 for further dilution. At this concentration and at a temperature of 25-30°, crystalline GA dissolves completely in water in 20-30 minutes with constant shaking. We made all dilutions with tap water which had been freed of chlorine by heating. In our experience, a solution of pC 4 can be kept in the refrigerator for quite a long time, but low concentrations should be prepared on the day of the experiment.

Voronezhskaya 76 corn served as our chief experimental material. In addition, other varieties were tried—Voronezhskaya 14, Sibiryachka, Beloyaroe millet, Rannyaya Zhemchuzhina and Pionerka-Severa—and they gave similar results, although individual differences were found.

TABLE 3

The Degree of Significance of the Difference in length Increase between Leaf Sections in Water (control) and in GA Solutions of pC 7.0, 6.7, and 6.0, with 10 Sections per Treatment (the number of cases for each level of significance is expressed as a percentage of the total number. For pC 7.0, this was 50 experiments, for pC 6.7 it was 18 experiments, and for pC 6.0 it was 54 experiments)

Degree of significance	No. of cases (%)		
	7.0	6.7	6.0
Significant	64	95	98
Probably significant	12	5	2
Insignificant	24	0	0

After a thorough washing, the seeds were soaked at 18-25° for 10-12 hours in tap water which had been freed of chlorine. After soaking, the grains were laid out, embryo downward, in flat cuvettes on damp filter paper. The cuvettes, which were covered with glass plates, were kept in the light until the appearance of rootlets 0.5-1 cm long, after which the grains were transferred to flats; the flats themselves represented small cuvettes with a network of parallel glass rods traversed by rubber tubes (Fig. 2 A). The flats were filled with water and filter paper was laid over the lattice, being perforated with small holes in rows lying along the glass rods. The swollen grains were placed over the openings and the flats placed in a dark room at 22-25°, although the temperature actually fluctuated from 18 to 28°, which only affected the growth rate of the seedlings.

For experiments we used seedlings in which the coleoptile had completed its growth and the first leaf had pierced through it and grown 1-5 cm beyond it.

Dissection of the seedlings was performed in light, care being taken to avoid direct sunlight. All seedlings from a given flat were cut at the base of the mesocotyl, placed on damp filter paper in large cuvettes, and divided into groups according to the length of the first leaf in such a way that the variation within a group should not exceed 5 mm. In order to obtain statistically equivalent treatment groups, composed of 12 seedlings each, the following procedure was employed. Seedlings in the group with the shortest leaf were distributed equally among all the treatment groups; seedlings in groups with successively longer leaves were treated in the same manner until each treatment group contained 12 seedlings. Groups in which the number of seedlings was less than the number of treatments were discarded. This method of selecting treatment groups made it possible to use most of the seedlings in a given flat, while the method of selecting treatment groups from seedlings with leaves of the same length would necessitate using only 20-30 % of all the seedlings. We recommend that a group number 12 seedlings in order to allow for the discard of the two most deviant values in the final analysis of the material.

After distribution of the seedlings into treatment groups, sections of a total length of 35 mm were prepared, 5 mm being mesocotyl and 30 mm being leaves included in the coleoptile. We employed this length because with shorter sections the growth is reduced and the relative error increased, and with longer sections technical difficulties arise. The seedlings were cut with a sharp knife, being laid out on millimeter-ruled paper covered with transparent film or on clean paper on which the proper distances (5 mm + 30 mm) had been marked with a pencil. The boundary between the coleoptile and the mesocotyl served as the point from which the measurements were made; leaves together with coleoptile were sectioned 30 mm above this boundary, and the mesocotyl 5 mm below it.

The sections were then placed mesocotyl downward, in small glasses 30 mm high with an internal diameter of 15-17 mm (see Fig. 2B). Each glass contained 12 sections, corresponding to one treatment group or a replicate. Before the sections were placed in the glasses, 1-2 ml of the solution to be tested, water (control) or a GA solution (standard), were poured in. The glasses were placed in a glass chamber which was then placed in a dark room at 22-25°.

Because of the low stability of solutions of pA near 7, the sections were measured the first and second days after the beginning of the experiment, and the values taken were those differing the most from the control. With more active solutions (pA 6 and higher) it was better to take measurements on the third or the fourth day, since the difference with the control was higher on these days.

Measurements were made with an accuracy of 1 mm using a ruler or millimeter ruled paper covered with transparent film. Only the first leaf was measured, taking the base of the coleoptile as the starting point. The length of the mesocotyl was not measured or taken into consideration.

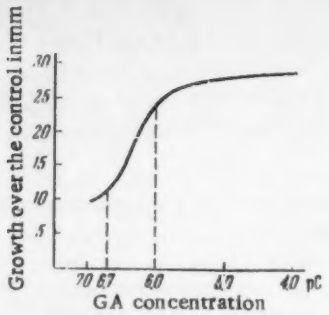

Fig. 3. GA activity curve for sections of leaves from Voronezhskaya 76 corn seedlings. The straightest portion of the curve, between pC 6.7 and pC 6.0, is indicated by dotted lines.

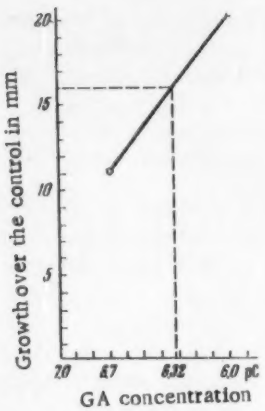

Fig. 4. An example of the determination of activity of an "unknown" solution by graphical interpolation in an experiment with four replicates of 10 sections each. Standard GA solutions of pC 6.7 and 6.0 gave average growth differences with the control of 11.1 mm and 20.4 mm. An "unknown" solution of GA, pC 6.6, gave growth increases of 16.1 mm. After interpolation (dotted lines), the activity pA of the solution turned out to be 6.32 ± 0.05, with a maximum deviation of 0.15 units.

An important feature of the results obtained was the statistical significance of the differences between control and active solution, or between active solutions themselves. To use for this a complex statistical treatment with all the pertinent factors is not sensible inasmuch as results of experiments in plant physiology do not in general completely fulfill the requirements for variational statistical analysis.

The statistical significance of the difference (ρ) between treatments may be determined by the following formula:

$$\rho = \frac{M_1 - M_2}{\sqrt{m_1^2 + m_2^2}},$$

where M_1 and M_2 represent the means of the section lengths in two treatments, and m_1 and m_2 the corresponding standard deviations for each treatment found according to the formula:

$$m = \pm \sqrt{\frac{\Sigma x^2}{n(n-1)}},$$

where x^2 is the sum of the squares of the deviations from the mean in a given treatment and n is the number of sections in each treatment (here, usually n = 10).

If $\rho \geq 3$, the difference between treatments is considered statistically significant; if $\rho \geq 2$ and < 3 it is probably significant, and if $\rho < 2$ it is insignificant.

Using this method, we determined for many cases with n = 10 the degree of significance of the difference in increase between the control (water) and GA solutions of pC 7.0, 6.7 and 6.0. The experiments performed in this way were made at different times with the varieties Voronezhskaya 76 and Siberyochka and at various temperatures (18-28°); in some experiments the temperature fluctuated as much as 5°. The results obtained are presented in Table 3.

As Table 3 shows, with n = 10 the difference in increase between the control and the pC 6.0 GA solution is almost always significant; at pC 6.7, the significance is also high, while at pC 7.0, results obtained in 24% of the cases are insignificant. In order to insure that differences will always be significant at this concentration, it would therefore, be necessary to increase the number of replicates several times. Very often, however, this is of no avail, since the lack of significant results is due in this case not to an increase in statistical error but to a decrease and even to a disappearance of differences with the control because of the rapid inactivation at pC 7.0 of which we spoke previously. We consider the minimum concentration (pC) of GA which gives results consistently significant at n = 10 to be 6.7. At all higher concentrations, results are always significant. We may also say, on the basis of a very large number of experiments, that when the absolute difference between the control and a given concentration exceeds 10 mm statistical treatment may be omitted, since differences of such a magnitude are always significant, or at least probably so. The demonstration of statistical significance of small differences between concentrations above 6.7 requires, of course, that the number of sections in an experiment be increased; the smaller the differences, the more replicate groups of 10 sections are used.

In contrast to our data of Table 3, which are based on a large number of experiments, the data of other workers are based only on individual experiments. It is not, of course, possible to judge the statistical significance of their results at low concentrations. In two of these studies [18, 26] a concentration (pC) 7.0 was not used, in one study [27] it yielded insignificant results, and in another [16] the difference obtained, 0.71 mm, was considered significant (!).

The assay described provides the means to detect unidentified gibberellin-like substances and also to evaluate quantitatively their activity by the method described in 1947 for auxins [25]. This method is analogous to the colorimetric determination of materials using suitable colored standards and differs from it only in that the measurement criterion is growth in comparison with the control instead of color intensity. The determination, by graphical interpolation on a curve constructed from standard solutions, is most simple to perform. Known concentrations of GA, from which the activity curve of Fig. 3 is constructed, serve as standards. Inspection of this curve shows that the straightest portion occurs between pC 6.7 and pC 6.0, which were taken as standards.

The growth in an experimental solution should be within the limits of growth increases established with the standard solutions. Then, by graphical interpolation , that concentration corresponding to the amount of growth obtained experimentally may be found, as shown in Fig. 4. Since the concentration found is not the actual concentration of the unknown, but only the equivalent in terms of growth activity, we designate it by the symbol pA. In order to make the determination more accurate, the number of replicate groups of 10 sections should be increased three to five times. Even so, the method cannot pretend to great accuracy inasmuch as a logarithmic error of 0.1 of a pC unit corresponds approximately to an error of 20 % in weight. Considering , however, that errors and losses during extraction and chromatography cannot be smaller, and also that the method described is the only one for evaluating quantitatively the activity of gibberellin-like substances in extracts from higher plants, one must be satisfied with this degree of accuracy. A discussion of extraction and chromatographic separation of these substances will be presented in a later communication.

The authors extend their thanks to Professor M. Kh. Chailakhyan, who was of great assistance in the successful completion of the work. They are also indebted to Professors F. F. Matskov and B. P. Ivanov for seeds of certain varieties of corn.

L. I. Shanina, a student at VSKhIZO, provided technical assistance.

SUMMARY

Sections of maize leaves taken with growing points and mesocotyls were employed as the test objects for detection of gibberellin activity. The reaction to gibberellins in such sections is much stronger than in leaves taken without growing points (Fig. 1). Moreover, indolacetic acid has only a slight inhibitory effect in this case. The sections are prepared in the light from 7–8 day old etiolated maize seedlings in which the first leaf had emerged from the coleoptile and grown outwards 1–3 cm. The total length of the sections is 35 mm (leaf 30 mm + mesocotyl 55 mm). From 10 to 12 sections are placed in small glasses 30 mm in height and 15–17 mm in diameter (Fig. 2), equal amounts (1–2 ml) of the solution investigated, or water (the control) being first poured into the glasses. The glasses with the sections are placed in a dark room at a temperature of 22–25° C and high humidity. The measurements are made after 2 or 3 days of incubation. A quantitative estimation of the activity is made by parallel experiments with gibberellic acid solutions and graphical interpolation (Fig. 4).

LITERATURE CITED

[1] B. B. Stowe and T. Yamaki, Ann. Rev. Plant. Physiol. 8, 181 (1957).

[2] P. W. Brian, Biological Revs. 34, No. 1, 37 (1959).

[3] R. A. Gray, Amer. J. Bot. 44, No. 8, 674 (1957).

[4] M. Kh. Chailakhyan, Bot. Zhurn. 43, 427 (1958).

[5] J. A. Lockhart, Physiol. Plantarum 11, 478 (1958).

[6] P. W. Brain and H. G. Hemming, Nature 179, No. 4556, 417 (1957).

[7] B. O. Phinney, C. A. West, M. Ritzel, and P. M. Neely, Proc. Nation. Acad. Sci. USA 43, No. 5, 398 (1957).

[8] J. P. Cooper, New Phytologist 57, No. 2, 235 (1958).

[9] T. H. Plummer and M. L. Tomes, Bot. Gaz. 119, No. 3, 197 (1958).

[10] P. W. Brain, H. G. Hemming and M. Radley, Physiol. Plantarum 8, No. 4, 899 (1956).

[11] J. Kato, Science 128, No. 3330, 1008 (1958).

[12] M. Kh. Chailakhyan and T. V. Nekrazova, Doklady Akad. Nauk SSSR 119, 4, 826 (1958)*

[13] J. Kato, Physiol. Plantarum 11, No. 1, 10 (1958).

[14] M. Radley, Nature 178, 1070 (1956).

[15] B. O. Phinney, C. A. West, M. Ritzel, and P. M. Neely, Proc. Nation. Acad. Sci. USA 43, No. 5, 398 (1957).

[16] M. Radley, Ann. Bot. 22, No. 87, 297 (1958).

[17] A. J. McComb and D. J. Carr, Nature 181, No. 4622, 1548 (1958).

[18] J. P. Nitsch, Bull. Soc. bot. France 105, No. 9-10, 479 (1958).

[19] G. M. Simpson, Nature 182, No. 4634, 528 (1958).

[20] A. Lang, J. A. Sandaval, and A. Bedri, Proc. Nation. Acad. Sci. USA 43, No. 11, 960 (1957).

[21] N. A. Krasil'nikov and M. Kh. Chailakhyan, et al., Doklady Akad. Nauk SSSR, 121, 4, 755 (1958)* 123, 6, 1124 (1958)*.

[22] J. MacMillan and P. J. Suter, Naturwissenchaften 45, No. 2, 46 (1958).

[23] C. A. West and K. H. Murashige, Plant Physiol. 33, Suppl. XXXVIII (1958).

[24] F. Kavanagh and N. R. Kuzel, Agric. and Food Chem. 6, 459 (1958).

[25] A. N. Boyarkin, Doklady Akad. Nauk SSSR 57, 2, 197 (1947); 59, 9, 1651 (1948).

[26] E. Galun, Experimentia 15, No. 5, 184 (1959).

[27] R. S. C. Aytoun, A. T. Dunn, and D. A. L. Seilor, Analyst 84, No. 997, 216 (1959).

[28] A. J. Vlitos and W. Meudt, Nature 180, No. 4580, 284 (1957).

[29] R. A. Fisher, Statistical Methods for Research Workers. (Lond. Fifth edition, 1934).

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A CHROMATOGRAPHIC METHOD OF DETERMINING HEXACHLOROCYCLOHEXANE IN PLANTS

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The adoption of hexachlorocyclohexane (GBH) as an agent for control of plant parasites has necessitated the study of its effect on the biochemical and physiological processes of cultivated plants. In connection with such study it was necessary to elucidate the problem of its entrance into plants and also to develop methods of analyzing microquantities of it.

We began a study of colorimetric methods of analysis, the most sensitive, and first tested those described in [1, 2].

In the methods described, GBH had been determined in the stomachs of curculionids. With certain changes, which we have already described [3], this method was used for determinations of GBH in plant tissues. Its sensitivity was, however, inadequate for this purpose.

Shekhter and Gorshtein [4] have suggested a method which is sensitive enough to determine GBH in quantities as low as 0.003 mg. They consider their method suitable for determination of GBH in plant tissues.

We attempted to analyze for GBH according to this method [4]. We were able to determine quantities as low as 2 μg, but analysis in a plant sample did not yield clear-cut and reproducible results. Very often even the analysis of a pure preparation was not succesful, notwithstanding the fact that we carefully followed the directions regarding reagent purity. None of the elaborations of this method [4] published subsequently was able to help us to make it yield satisfactory results.

We solved the problem of separation and analysis of microquantities of GBH in plant material in the range 3-5 μg by the use of paper chromatography.

In 1952, Mitchell [5] described a chromatographic method of separating the isomers of technical grade HCCH We tried to repeat his experiment. We were unable to separate the isomers as he had done. Only with comparatively large quantities —50 μg and more — were two or three spots obtained on the chromatograms; with smaller quantities only one spot was obtained. Fortunately, however, it was possible to separate the isomers in toto from a plant extract containing GBH and organic compounds of the plant.

In chromatographing we used the following reagents and glassware: 1) chromatograph paper from the Leningrad factory; 2) cylindrical glass vessel 33-34 cm high with an internal diameter of 18-19 cm; a glass hook projects from the inner side of the ground cover; 3) a glass atomizer about 100 ml in volume; 4) a fraction of petroleum ether boiling at 60-65°; 5) a fraction of ligroin boiling in the range 60-80°, used as a mobile solvent. Mitchell used a fraction consisting of octanes; 6) ethyl ether; 7) acetic anhydride as the nonmobile solvent; 8) a normal solution of potassium hydroxide in methyl alcohol; 9) 0.05 N silver nitrate in dilute nitric acid (1 : 3).

Before chromatography the paper should be washed with distilled water and dried in air. Both fractions of ligroin should be first purified several times with sulphuric acid. Each time, 1 liter of ligroin should be shaken in a separatory funnel with 20-30 ml concentrated, chemically pure sulphuric acid until the acid is colorless.

The analysis was carried out in the following manner: strips of paper 30-32 cm long and not more than 16 cm wide were cut. The width of a strip depended on the number of spots to be made with the solution to be

analyzed. All operations with the paper should be performed with rubber gloves or with pincers. Otherwise spots are produced at the points of contact with the paper.

The wheat tissue was ground in a mortar and then placed in a flask containing a ligroin fraction with boiling point at 65°. On the following morning the extract was decanted into a separatory funnel and purified by shaking with 5-10 ml of concentrated sulfuric acid. Purification was carried out two or three times. Each shaking was for 5-10 minutes. This purification is necessary for the removal of colored substances which interfere with the formation of clearly visible spots characteristic of GBH.

The extract was then reduced to a volume of 0.4-0.6 ml and the residue spotted on the paper witha pipette in 0.1 ml amounts. The flask was twice rinsed with 0.2-0.4 ml of ligroin (b.p. 65°) and the washing applied to the same spot in the same manner as for the extract. In placing the drop on paper it is necessary to take care that the spot diameter not exceed 1 cm. To hasten the evaporation of the solution on paper a stream of air from a blower or ventilator can be used. Spots should not be less than 2.5 cm from the side of the paper. It is possible to spot several different extracts on one strip of paper. These spots should be not less than 3.0 cm from the lower edge of the paper and from each other. At the beginning of the analysis a line should be drawn, 1.2 cm from the lower edge and the required number of spots indicated.

After application of the extracts to the paper strip, it should be sprayed from a glass atomizer with a mixture of nine parts of ethyl ether and one part of acetic anhydride (according to volume) and lowered into the chromatography chamber. An amount of developing solution, ligroin boiling in the range 60-80° saturated with acetic anhydride (six parts of acetic anhydride to 100 parts of ligroin), which will be enough to cover the lower edge of the paper strip, attached to the cover of the chamber, to a depth of 1.2 cm is poured into the chamber.

The paper remained immersed in the solution for about 2 hours. During this time the front moved 20-25 cm from the lower edge; it should never reach the upper edge. GBH was localized 17-20 cm from the lower edge.

This is the pattern obtained with this paper and using ligroin. It is possible that under other conditions the picture would be different.

After 2 hours, the paper was removed from the chamber and dried in air. After about an hour, the paper was sprayed with a normal solution of potassium hydroxide in methanol. Spraying should be carried out in such a way that the entire surface of the paper is evenly covered with the liquid. The paper was then left in an oven at 130-133° for 30 minutes. On the following morning it was sprayed with a 0.05 N solution of silver nitrate in dilute nitric acid (water: acid, 1:3) and then placed in the sun. After a few minutes, characteristic spots appeared over the points of application of extracts or solutions containing GBH.

The paper should not be sprayed with a silver nitrate solution immediately after heating, since a dark background is produced which hinders identification of spots.

Where the plant extract contained no GBH, no spots were produced provided the extract had been initially purified with sulfuric acid.

Examination of the sensitivity of the method showed the following: In the analysis of solutions of GBH in ligroin , 0.003 mg gave clearly visible spots. If a given amount of GBH was added to an extract of 1-5 g of wheat or grass tissue, even then 0.003 mg GBH gave a clearly distinguishable spot (0.002 mg could not always be detected). It is possible to estimate the amount of GBH in the sample analyzed by the color intensity of the spot.

In a study of the entrance of GBH into the root system, wheat seeds covered with a 12% GBH dust (1 kg dust per cwt of seeds) were sown.

Analyses showed that eight days after sprouting the wheat contained 2 μg GBH per gram green tissue, and 21 days after sprouting there were 3 μg per gram. This is equal to 0.0002 to 0.0003 %. Comparing these data with data obtained using the quantitative colorimetric method [3], we see that even there we found from 0.00025 to 0.0004 % GBH in wheat.

SUMMARY

A chromatographic method for determination of microamounts of hexachlorocyclohexane in plant tissues is suggested.

The method can be used not only as a qualitative but also to a certain degree as a quantitative method of analysis of hexachlorocyclohexane in plants.

LITERATURE CITED

[1] G. Armstrong, F. R. Bradbury, and H. Standen, Ann. Appl. Biol. vol. 38, No. 3, 555 (1951).

[2] F. R. Bradbury and H. Standen, J. Soc. Food Agri. 5, 252 (1954).

[3] L. M. Fukel'man, Trudy Mold. Stantsii Zaschity Rastenii 2 (1957).

[4] Analyt. Chem. 24, 544 (1952).

[5] J. Assoc. Offic. Agric. Chem. 35, No. 4, 920 (1952).

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A DRY WIND APPARATUS

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Not infrequently during the study of plant drought resistance the need arises to investigate the effects of dry wind. For this purpose a special apparatus is required to produce natural dry winds. For this reason, at the Artificial Climate Station of the Timiryazev Institute of Plant Physiology , Academy of Sciences, USSR, a special air-conditioning apparatus has been built which creates a temperature of 40-45°, relative humidity of 20-30 % and a wind speed of 5-10 meter/sec. in a greenhouse chamber having an area of 23 m^2 and a volume of 92 m^3.

The greenhouse air-conditioning apparatus operates as shown in Fig. 1. The air circulating from the greenhouse is sucked by an exhaust fan through a grill into an air duct. Part of this air is discarded to the outside and the remainder is channeled into the mixing chamber where it is mixed with fresh outside air. The mixture of recirculated and outside air flows through an air cooler, passes through the first preheater, a scrubbing chamber, an intake fan, through a second preheater, a rehumidifing chamber and into the greenhouse. The air flows into the greenhouse through an intake duct with a 15·82 cm opening. Air passing through separate parts of the apparatus can be cooled or heated, dried or moistened.

Thermoregulators placed after the scrubber maintain the required dew-point temperature by regulating a

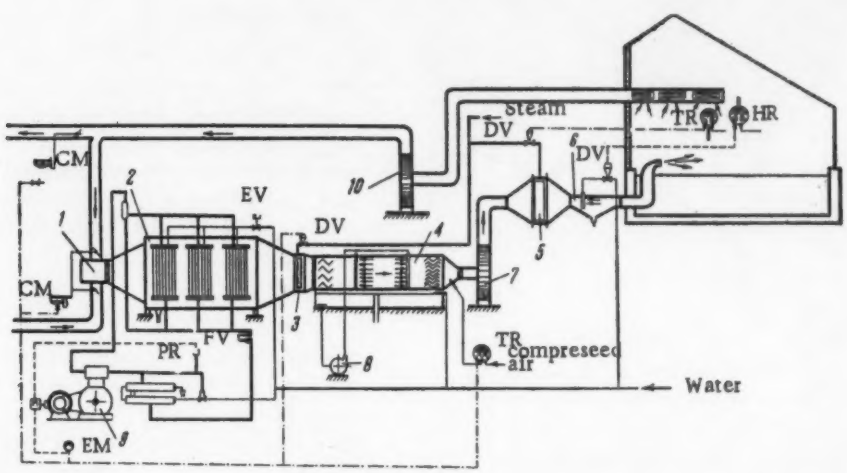

Fig. 1. Diagram of the greenhouse air-conditioning apparatus. 1) Mixing chamber; 2) air cooler; 3) first preheater; 4) humidifying chamber; 5) second preheater; 6) rehumidifying chamber; 7) air intake; 8) circulating pump; 9) freon regriferator; 10) exhaust fan; TR) thermoregulator; HR) humidity regulator; PR) pressure regulator; EM) electrocontact manometer; FV) float valve; CM) control mechanism; DV) diaphragm valve; EV) electromotor valve.

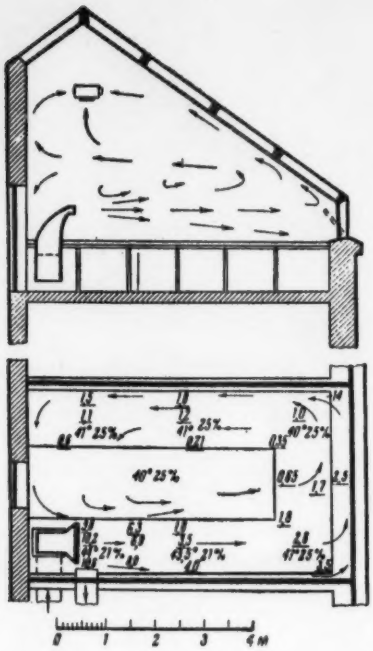

Fig. 2. Air velocity, temperature and humidity in the dry wind apparatus. Speed (m/sec) underlined; arrows indicate direction of air movement; temperature (°C); relative humidity (%).

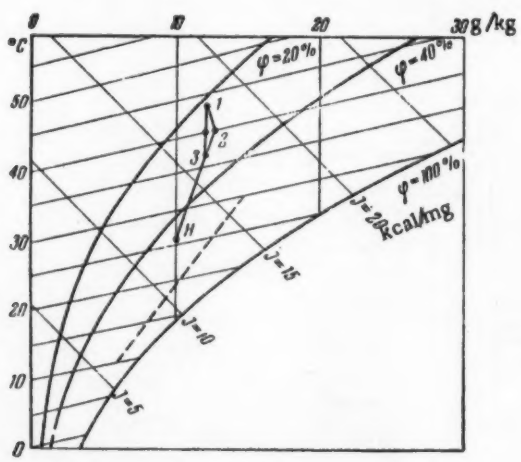

Fig. 3. Change in condition of the air in the apparatus. 1) Entering greenhouse; 2) leaving greenhouse; 3) in mixing chamber; O) outdoors; dashed line is the observed Moscow average diurnal air temperature and humidity; setting of apparatus—air temperature 40°, relative humidity 25%.

damper in the air exit from the greenhouse and one at the entrance of the mixing chamber, and thermoregulators also govern the air cooler and the fan which forces steam into the first preheater. Thermoregulators in the greenhouse govern a valve which forces steam into the second preheater to maintain a specified air supply temperature. Humidity regulators in the greenhouse control a valve which admits water to the rehumidifying chamber.

Every hour a volume of air 45 times the volume of the greenhouse is circulated. The air emerges from the delivery duct with a speed of 9.8-10.6 m/sec, although the speed of the current beneath the benches is lower. In other parts of the greenhouse the air beneath the benches moves with a speed of 0.2-0.6 m/sec. The variations in air speed and direction in the greenhouse are shown in Fig. 2.

Only a part of the air conditioning apparatus operates during a specified regime in the greenhouse: the mixing chamber with the automatic air dampers, the second preheater, the intake and exit fans and the thermoregulators. The other parts of the air-conditioning apparatus are shut off. The air flows through in the following way: Recirculated air is mixed with outside air, the mixture is preheated to the required temperature and is blown into the greenhouse. All of these operations are automatically controlled by thermoregulators.

Fig. 2 shows the air temperature and relative humidity 62 cm above the benches in the greenhouse with the apparatus set at a temperature of 40° and a relative humidity of 25%. The specified temperature is maintained within ± 1° and the humidity ± 2%. This apparatus has been used for 2 seasons (1957-1958) by the laboratory of Drought Resistance directed by P. A. Genkel' for studying the effect of dry winds on plants.

The process of changing air condition in the greenhouse is depicted in the diagram (Fig. 3). If the temperature of the air entering the greenhouse is decreased 4°, the relative humidity is increased 4%, and the specific humidity is increased 0.3 g/kg. The humidity of the air does not increase appreciably inside the greenhouse.

For experimental purposes, the greenhouse monitoring devices can be adjusted to simulate natural dry wind conditions existing during a growing season. Required air temperatures of

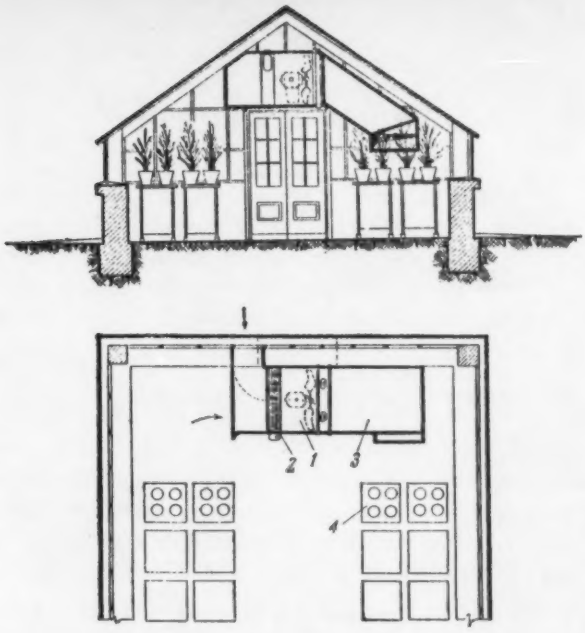

Fig. 4. Greenhouse with dry wind apparatus. 1) Heating-ventilating assembly; 2) double air valve with electrically driven mixing chamber; 3) intake hood; 4) truck with plants.

40-45° and relative humidities of 20-30 % can be obtained in such a greenhouse if the specific humidity outside does not exceed 10-11 g/kg. The dry wind apparatus operates in the following way. Air from the greenhouse and fresh outside air sucked in by the fans is mixed, preheated and forced at the required speed through the intake duct into the greenhouse. Surplus air exits through the ventilators which are opened as needed.

A dry wind apparatus operating according to this scheme is depicted in Figure 4. It can be built from a number of parts and devices being commercially produced. The apparatus consists of a heating-ventilating assembly, a mixing chamber with automatically controlled fans, an intake duct, and thermoregulators. The thermoregulator in the mixing chamber maintains the mixture of outside and recirculated air at the specified temperature which thus permits the maintenance of required humidity. A thermoregulator in the greenhouse maintains the specified air temperature. Manufacture of the heating-ventilating assembly, the dimensions of the intake duct and the air dampers depend upon the dimensions of the greenhouse.

This same apparatus can also be used for heating the greenhouse during winter or ventilating it during the summer. The output of the heater should compensate for heat loss by the greenhouse during winter; the output of the ventilator fans should supply the required volume of fresh air during the summer.

SUMMARY

At the Artificial Climate Station of the Timiryazev Institute of Plant Physiology an apparatus has been built which maintains a temperature of 40-45°, relative humidity of 20-30 %, and a wind speed of 5-10 m/sec in an air-conditioned greenhouse with an area of 23 m^2 and a volume of 92 m^3. The volume of air circulated per hour is 45 times the volume of the greenhouse. The temperature is maintained to within ± 1°C and humidity ± 2 %.

The described automatic dry wind apparatus for greenhouses can be constructed from commercially produced parts.

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CURRENT EVENTS

PLANT PHYSIOLOGY IN AZERBAIDZHAN

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Plant physiology in Azerbaidzhan is a comparatively young science. No physiological investigations were conducted in Azerbaidzhan before the revolution. The first work in physiology began in 1928 when the All-Union Institute of Applied Botany, Genetics and Selection conducted investigations on the water regime and drought resistance of wild and cultivated plants grown in the vicinity of Baku and in its district [1].

In 1921 the Botanical Institute of the Academy of Sciences USSR did much work on the ecological and physiological characteristics of cork oak in the Lenkoranskii district of the Azerbaidzhan SSR [2]. These investigations showed that the cork oak is a photophilic plant with high drought resistance and is able to transpire at a high rate when the soil moisture supply is sufficient.

During 1930 co-workers of the Department of Plant Physiology of the Upper School and the Azerbaidzhan Agricultural Institute worked on problems connected with the physiology of crop plants. D. A. Shutov and L. Kh. Kara Murza endeavored to throw light upon the processes occurring during the initial growth stages of rice [3]. Jointly with V. N. Naugol'nykh they also conducted studies on the water culture of cotton [4].

With the organization of the Azerbaidzhan section of the Trans-Caucusus Branch of the Academy of Sciences USSR (1932) and its reorganization into the Azerbaidzhan Branch of the Academy of Sciences (1934) research in physiology was somewhat expanded.

With the material support of the Trans-Caucusus Branch of the Academy of Sciences USSR and the Department of Plant Physiology of the Azerbaidzhan Agricultural Institute, these workers studied a number of physiological problems, among them the salinity tolerance of cotton, the resistance of cotton to soil drought, and the effect of harmful salts on glasswort (Salicornia) [5]. These investigations made possible an explanation of the relationship between injurious salts, soil drought and cotton production.

In 1936 the Azerbaidzhan Branch of the Academy of Sciences USSR organized an Institute of Botany which set up a Department of Plant Physiology. With the organization of this Institute , work in plant physiology was expanded still further, and in 1936 the Department of Plant Physiology investigated the mineral nutrition and salt tolerance of cotton [6]. Somewhat later, in 1937, D. A. Shutov, director of the Department of Plant Physiology, and his co-workers studied the winter hardiness of evergreen subtropical woody species in the Baku climate. These experiments disclosed the nature of the plant stresses resulting from both low temperature and winter drought in the Baku climate [7].

In subsequent years, 1940-41, D. A. Shutov studied the water regime of upland xerophytes, halophytes, and, among the subtropical crops, the Eucalyptus [8].

During World War II, because of a critical requirement for vitamins, the Department of Plant Physiology investigated the vitamin content of the leaves of wild and cultivated plants [9]. This work showed that it is possible to develop Azerbaidzhan plants very rich in vitamins and to forecast their sap yield, and more appropriate methods for manufacturing vitamin preparations were recommended.

Because of an urgent need for food stuffs during these years , the workers of the Institute of Botany worked out a method for preserving potato tops [10]. During the three year period, 1940-42, the external root nutrition of potatoes [11] and cotton [12] was studied. The Institute of Botany has been working for a number of years on the translocation of certain elements in the cotton plant [13].

Research in physiology by the Institute of Botany was appreciably expanded in 1945 when the Branch of the Academy of Sciences USSR was reorganized into the independent Academy of Sciences, Azerbaidzhan SSR.

The Department of Plant Physiology and Biochemistry began studies on both physiological and biochemical problems. Of the physiological problems especially great importance was attached to those involving plant mineral nutrition.

The effect of mineral nutrition on plant development and aging [14] and the effect of mineral fertilizers on the oxidation-reduction processes in plant tissues [15] were studied by this Department during 1945-46. The mineral nutrient requirement of cotton during different developmental stages was investigated with the aim of increasing yields [16].

To a great extend aimed at increasing the yield of the principle crops (cotton, alfalfa, etc.) through the use of elemental mineral fertilizers, quite a bit of attention has been devoted to the question of using microelements, especially the microelement manganese, which, since 1948, has been produced by the Frunze factory as a commercial waste product in the form of manganese sulfate.

Under both greenhouse and field conditions on different soils and under a wide variety of field conditions in the kolkhozes and sovkhozes* of the Azerbaidzhan SSR, for three years after 1949, physiological investigations were made on the effect of manganese sulfate as a microfertilizer in relation to the growth, development, and yield of cotton and alfalfa [17, 18]. These experiments showed that manganese sulfate increases cotton yields on the average 15-19.8 % and alfalfa seed yields 30-35 %. The Department of Plant Physiology and Biochemistry has drawn up instructions for the use of manganese sulfate as a microfertilizer on cotton and alfalfa [19]. The effect of the microelements boron, manganese, and other microfertilizers on the growth, development, and yield of cotton and grain crops has also been investigated by the Department of Plant Physiology of Azerbaidzhan State University and the Institute of Agriculture of the Academy of Sciences, Azerbaidzhan SSR [20].

In the Department of Plant Physiology of the Institute of Botany physiologic investigations have taken an ecological direction.

The need to develop in this direction was dictated by the specific conditions of Azerbaidzhan with its abruptly droughty climate and soil saltiness which required an investigation of the physiological nature of the effect of these unfavorable environmental factors upon plants. The investigations conducted have allowed us to come closer to an understanding of drought itself and the effect of soil salts, both of which restrict plant growth, and to contemplate a concrete approach to combating them.

A study of the salt tolerance of potato begun in 1943 was concluded in 1947 [21].

Since 1952 a directed increase in the salt tolerance of cotton using the method worked out by P. A. Genkel' has been studied in the greenhouse and under a broad range of field conditions. These investigations showed that if, prior to sowing, cotton seeds were exposed to soils made weakly and moderately salty with chlorides by Genkel's method, the plants produced higher yields than control plants [22].

The physiology of the drought tolerance of woody species of Apsheron was studied in the Department of Plant Physiology of the Institute of Botany from 1953-1955 [23]. With the aim of speeding up the maturity of cotton bolls the preharvest removal of cotton leaves has been investigated under production conditions on the Mugan in the Agdam and Sal'yany districts [24].

Investigating biochemists of the Department of Plant Physiology of the Institute of Botany have studied the influence of temperature on the synthesis activity of proteases [25] and trends in the activity of carbohydrate enzymes [26].

Because of expansion in the tea-raising area, studies of the biochemistry of tea have evolved in the Lenkoranskii Tea Sovkhoz [27].

For several years (1948, 1949) the trends of enzyme activity and the rate of carbohydrate accumulation in different varieties were studied in relation to the degeneration of potatoes [28].

In order to deepen knowledge of the vital processes, since 1954, the Department of Plant Physiology of the Institute of Botany has been studying the relation of external factors to anatomical changes in plants [29].

* Kolkhoz) a collective farm; sovkhoz) state farm.—Publisher.

In conclusion, it ought to be observed that physiological work in Azerbaidzhan today is still expanding and has a scope greater than that in the other Republics. For example, the Department of Plant Physiology of the Institute of Botany on its septennial prepared a detailed paper on the physiology of drought and salt tolerance of forage plants (B. Z. Guseinov, Z. S. Azizbekova, Z. Yu. Mamedova) which is valuable in our efforts to firm-up fodder supplies and increase the animal production of the Republic.

In the State University and the Institute of Agriculture a number of studies have been made of the translocation of mineral elements in the plant and the mechanism for this translocation has been revealed (M. G. Abutalybov et al). A number of studies have also been made of the effect of microelements on photosynthesis, winter-hardiness, and the salt tolerance of plants.

As a happy event in the affairs of the Republic we ought to observe the publication of Volume One of a textbook on plant physiology in the Azerbaidzhanian language and the preparation for publication of Volume Two of this textbook compiled by Professor M. G. Abutalybov.

We should note as a great achievement in the task of preparing highly-qualified staff members from among native Azerbaidzhanians the publication of a book on plant anatomy and physiology compiled by B. Kh. Tutayuk in the Azerbaidzhanian language for students of the higher schools and the University.

At present, thanks to the concern of the party and the government, the laboratories of plant physiology of a number of scientific research institutes in the Republic have the very latest equipment. Radiobiology laboratories have been opened in the Department of Plant Physiology of the Institute of Botany, Academy of Sciences, Azerbaidzhan SSR, in the Institute of Agriculture of the Ministry of Agricultural Economics, and in the Department of Plant Physiology of Azerbaidzhan State University.

A number of studies of the effect of ionized radiation on physiological processes in plants, assimilation mechanisms, the translocation of nutrient materials and on other vital processes have been made in these laboratories.

Scientific research work in the radiobiology laboratories of these institutes makes possible the posing of broader and deeper physiological problems and more detailed study of physiological processes occurring in plants.

LITERATURE CITED

[1] C. P. Kuz'min, Trudy po Priklad. Botan. Genet. i Selecktsii 23, No. 2, 293 (1930).

[2] L. E. Pravdin, Éxplt. Botan. 3, 151 (1937).

[3] D. A. Shutov and L. Kh. Kara Murza, Botan. Sb. Gos. n. -i Inst. No. 1, 85 (1932).

[4] D. A. Shutov and L. Kh. Naugol'nykh, Material on Ezperimental Work on the Mugan, No. 2, 32 (1936) [in Russian].

[5] D. A. Shutov, Trudy Azerb. Filiala Akad. Nauk SSSR 15, 108 (1936).

[6] M. G. Abutalybov, Trudy Azerb. Filiala Akad Nauk SSSR 9, 5 (1940).

[7] D. A. Shumov, S. G. Él'gort, and M. M. Elfimova, Trudy Azeb. Filiala Akad Nauk SSSR 6, 5 (1939).

[8] D. N. Shutov, Trudy Inst. Azerb. Filiala Akad. Nauk SSSR 12, 137 (1940).

[9] A. L. Gasanov, Authors Abstract of Candidates Dissertation, Botanical Institute (Baku, 1948) [in Russian].

[10] N. G. Abutalybov, Z. S. Azizbekova, Z. G. Gaibov and A. L. Gasanov, Izv. Azerb. Filiala. Nauk SSSR No. 4, 64 (1942).

[11] Z. S. Azizbekova, Izv. Azerb. Filiala Akad. Nauk SSSR 1, 39 (1943).

[12] M. G. Abutalybov, Trudy Azerb. Filiala Akad. Nauk SSSR 9, 5, 1 (1940).

[13] M. G. Abutalybov, Trudy Azerb. Filiala Akad. Nauk SSSR 9, 55 (1938).

[14] M. G. Abutalybov and E. G. Gaubov. Doklady Akad. Nauk Azerb SSR 2, 108 (1946).

[15] B. Z. Guseinov, Doklady Akad. Nauk Azerb SSR 2, 76 (1946).

[16] M. G. Abutalybov, Trudy Azerb. Filiala Akad. Nauk SSSR 6, 37 (1939).

[17] G. A. Aliev and B. Z. Guseinov, Doklady Akad. Nauk Azerb SSR 6, 10 (1950).

[18] A. Kh. Tagi-Zade, Author's Abstract of Doctor's Dissertation (Baku, Azgosuniversitet, 1957) [in Russian].

[19] Instructions for Using Manganese Sulfate Waste on Cotton and Alfalfa (Izd. AN Azerb. SSR, 1951) [in Russian].

[20] M. G. Abutalybov, Transaction of the All-Union Conference on Microelements, Latv. SSR 1956) [in Russian].

[21] Z. S. Azizbekova, Author's Abstract of Candidates Dissertation, Botanical Instute (Baku, 1946) [in Russian].

[22] P. A. Genkel' and Z. S. Azizbekova, Fiziol. Rast. 2, 90 (1955).

[23] Z. S. Azizbekova, Trudy Inst. Botan. AN Azerb. SSR 22, 36 (1936).

[24] M. R. Rustambekov and Z. Yu. Mamedova, Trudy Inst. Botan. AN Azerb SSR 16, 55 (1952).

[25] M. R. Rustambekov, Izv. Akad. Nauk Azerb SSR, No. 7, 17 (1947).

[26] B. Z. Guseinov and M. G. Abutalybov, Izv. Akad. Azerb SSR, No. 9, 3 (1947).

[27] A. D. Kerimov and A. G. Imanova, Doklady Akad. Nauk Azerb SSR 7, 279 (1951).

[28] A. B. Kadymova, Trudy Inst. Botan. 19, 5 (1955).

[29] A. M. Gasanov, Author's Abstract of Cadidates Dissertation, Botanical Institute (Baku, 1958) [in Russian].

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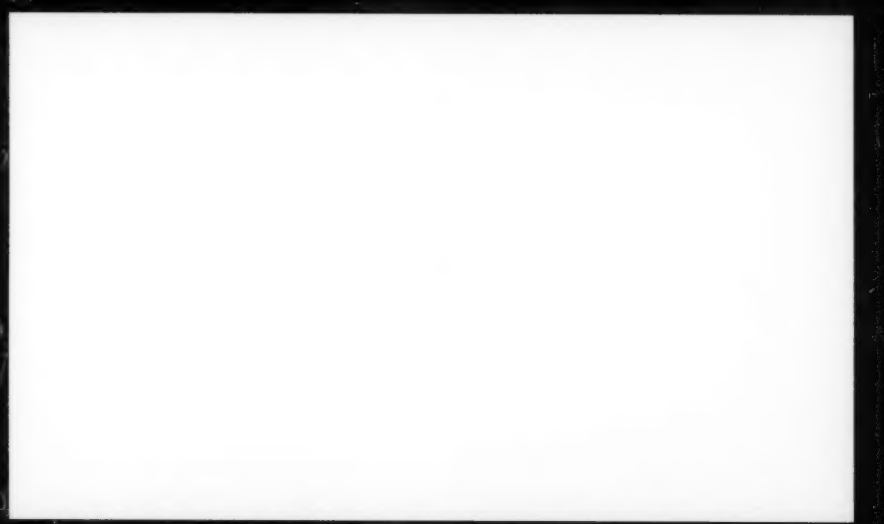

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